PATENT
Customer Number: 22,852
Attorney Docket No. 09367.0044-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Han-Jie Zhou et al.	)	Group Art Unit: 1614
	)	
Application No.: 10/527,540	)	Examiner: Not Yet Assigned
	)	
Filed: March 11, 2005	)	Confirmation No.: 5233
	)	
For: COMPOUNDS, COMPOSITIONS, AND METHODS	)	

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

PETITION FOR FILING DECLARATION UNDER 37 C.F.R. § 1.47(a) - NON-SIGNING INVENTOR

Pursuant to 37 C.F.R. § 1.47(a), Applicants hereby petition that the attached Declaration, executed by inventor Han-Jie Zhou be accepted by the U.S. Patent and Trademark Office on behalf of himself and non-signing inventor Andrew McDonald.

The pertinent facts concerning Dr. McDonald's refusal to join this application are set forth in the attached Declaration of Lauren L. Stevens, Ph.D. Dr. McDonald's last known work address is: ThinkEquity Partners LLC, 600 Montgomery Street, San Francisco, CA 94111, and his last known residence is 9 Barrow Street, Apt. 6D, New York, New York 10014-3864. His last known phone number is 415-249-2907.

Please grant any further extension of time required to enter this response, and charge any additional required fees to Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Date: January 3, 2007

By: _____
Lauren L. Stevens
Reg. No. 36,691

CERTIFICATE OF EXPRESS MAILING

Express Mail Label No.: EV 860820574 US

I hereby certify that this correspondence is being deposited with the United States Postal Services "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10, addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date below.

Date: **January 3, 2007**

Signed: _____
Linda Phillips

PATENT
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Attorney Docket No. 09367.0044-00000

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Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF LAUREN L. STEVENS, PH.D.

I, Lauren L. Stevens, Ph.D., do hereby make the following declaration:

1. I am a patent attorney employed by Finnegan, Henderson, Farabow, Garrett, & Dunner, LLP.

2. On March 11, 2005, I filed the subject patent application on behalf of Cytokinetics, Inc. The inventors are Han-Jie Zhou and Andrew McDonald.

3. On July 15, 2005, the undersigned sent a letter to Dr. Andrew McDonald at his current place of employment, ThinkEquity Partners LLC, 600 Montgomery Street, San Francisco, CA 94111, requesting Dr. McDonald to review the enclosed copy of the

application as filed and sign and date the Declaration and Power of Attorney and Assignment. The letter was sent by Federal Express. Copies of the letter, application, Declaration and Power of Attorney, Assignment, and the Federal Express delivery notice, that shows delivery at Dr. McDonald's work address on July 18, 2005, are attached at Tab 1.

4. On October 13, 2005, the undersigned sent another letter to Dr. McDonald at his current place of employment, ThinkEquity Partners LLC, 600 Montgomery Street, San Francisco, CA 94111, again requesting Dr. McDonald to review the enclosed copy of the application as filed and sign and date the Declaration and Power of Attorney and Assignment. The letter was sent by Federal Express. Copies of the letter and the Federal Express delivery notice, that shows delivery at Dr. McDonald's work address on October 14, 2005, are attached at Tab 2. (Copies of the application, Declaration and Power of Attorney, and Assignment were also enclosed with the letter to Dr. McDonald, and are the same as shown in Tab 1.)

5. On September 29, 2006, the undersigned sent a third letter (letter dated September 28, 2006) to Dr. McDonald at his current place of employment, ThinkEquity Partners LLC, 600 Montgomery Street, San Francisco, CA 94111, again requesting Dr. McDonald to review the enclosed copy of the application as filed and sign and date the Declaration and Power of Attorney and Assignment. The letter was sent by Federal Express. Copies of the letter and the Federal Express delivery notice, that shows delivery at Dr. McDonald's work address on October 2, 2006, are attached at Tab 3. (Copies of the application, Declaration and Power of Attorney, and Assignment were also enclosed with the letter to Dr. McDonald, and are the same as shown in Tab 1.)

6. On September 29, 2006, the undersigned sent the same letter (letter dated September 28, 2006) to Dr. McDonald at his residence, 187 Elsie, San Francisco, CA 94110, again requesting Dr. McDonald to review the enclosed copy of the application as filed and sign and date the Declaration and Power of Attorney and Assignment. The letter was sent by Federal Express. Copies of the letter and the Federal Express delivery notice, that shows delivery at Dr. McDonald's residence on October 2, 2006, are attached at Tab 4. (Copies of the application, Declaration and Power of Attorney, and Assignment were also enclosed with the letter to Dr. McDonald, and are the same as shown in Tab 1.)

7. On December 7, 2006, the undersigned sent a fourth letter to Dr. McDonald at his current place of employment, ThinkEquity Partners LLC, 600 Montgomery Street, San Francisco, CA 94111, again requesting Dr. McDonald to review the enclosed copy of the application as filed and sign and date the Declaration and Power of Attorney and Assignment. The letter was sent by Federal Express. Copies of the letter and the Federal Express delivery notice, that shows delivery at Dr. McDonald's work address on December 8, 2006, are attached at Tab 5. (Copies of the application, Declaration and Power of Attorney, and Assignment were also enclosed with the letter to Dr. McDonald, and are the same as shown in Tab 1.)

8. On December 7, 2006, the undersigned sent the same letter to Dr. McDonald at his residence, 187 Elsie, San Francisco, CA 94110, again requesting Dr. McDonald to review the enclosed copy of the application as filed and sign and date the Declaration and Power of Attorney and Assignment. The letter was sent by Federal Express. Copies of the letter and the Federal Express delivery notice, that shows

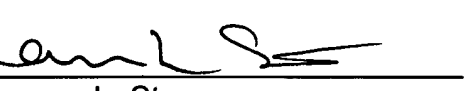
delivery at Dr. McDonald's residence on December 8, 2006, are attached at Tab 6. (Copies of the application, Declaration and Power of Attorney, and Assignment were also enclosed with the letter to Dr. McDonald, and are the same as shown in Tab 1.)

9. On December 7, 2006, the same letter and enclosures were e-mailed to Dr. McDonald at his work e-mail address, amcdonald@thinkequity.com. Copies of the letter, e-mail transmittal, and delivery notification that the e-mail message was successfully relayed to the recipient are attached at Tab 7. (Copies of the application, Declaration and Power of Attorney, and Assignment were also enclosed with the letter to Dr. McDonald, and are the same as shown in Tab 1.)

10. On December 11, 2006, it was brought to our attention that Dr. McDonald had just moved his residence. We, therefore, sent the December 7, 2006, letter to him by Federal Express to his current residence, 9 Barrow Street, Apt. 6D, New York, New York 10014-3864. Copies of the letter and the Federal Express delivery notice, that shows delivery at Dr. McDonald's current residence on December 12, 2006, are attached at Tab 8. (Copies of the application, Declaration and Power of Attorney, and Assignment were also enclosed with the letter to Dr. McDonald, and are the same as shown in Tab 1.)

11. Based on the fact that Dr. McDonald has not responded to any of these letters, it is my opinion that Dr. McDonald is refusing to sign.

Date: January 3, 2007

By: 

Lauren L. Stevens
Reg. No. 36,691

From: TrackingUpdates@fedex.com
To: <barnetts@finnegan.com>
Date: 7/18/05 2:57PM
Subject: FedEx Shipment 791678139787 Delivered

This tracking update has been requested by:

Name: 'not provided by requestor'

E-mail: 'not provided by requestor'

Our records indicate that the following shipment has been delivered:

Tracking number:	791678139787
Reference:	09367.0999-00000
Ship (P/U) date:	Jul 15, 2005
Delivery date:	Jul 18, 2005 14:01 PM
Sign for by:	Signature Release on file
Service type:	FedEx Standard Overnight
Packaging type:	FedEx Envelope
Number of pieces:	1
Weight:	2.0 LB

Shipper Information	Recipient Information
Palo Alto	San Francisco
CA	CA
US	US

Special handling/Services:
Deliver Weekday

Please do not respond to this message. This email was sent from an unattended mailbox. This report was generated at approximately 4:35 PM CDT on 07/18/2005.

To learn more about FedEx Express, please visit our website at fedex.com.

All weights are estimated.

To track the status of this shipment online, please use the following:
https://www.fedex.com/fedexiv/us/findit/nrp.jsp?tracknumbers=791678139787&language=en&opco=FX&clientype=ivpodalrt

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Thank you for your business.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

Stanford Research Park ▪ 700 Hansen Way ▪ Palo Alto, CA 94304-1016 ▪ 650.849.6600 ▪ Fax 650.849.6666
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LAUREN L. STEVENS, PH.D
650.849-6614
Lauren.Stevens@finnegan.com

July 15, 2005

Andrew McDonald, Ph.D.
ThinkEquity Partners LLC
600 Montgomery Street
San Francisco, CA 94111

VIA FEDERAL EXPRESS

Cytokinetics, Inc. Patent Application

Dear Andrew:

We are currently prosecuting an application for Cytokinetics, Inc. in the United States. We have enclosed certain documents for filing in the U.S. Patent and Trademark Office that require your signature.

The enclosed documents are the confidential and proprietary property of Cytokinetics, Inc. Consequently, the enclosed documents should not be copied or disclosed to anyone; however, you may retain copies of the application, the signed Declaration and Power of Attorney, and Assignment of Patent Application provided they are kept confidential and not disclosed to anyone.

Because you are a co-inventor of this application, we require your signature on the enclosed forms. Please review the enclosed copy of the application as filed, and sign and date all the forms as indicated.

Once you have reviewed and signed the enclosed documents at the indicated locations, please return all of the enclosed documents to us using the enclosed prepaid Federal Express envelope and address label.

To comply with U.S. Patent and Trademark Office rules, please let us know of any information and materials, including any prior art, that you are aware of that would be material to the examination of these applications. Information is considered material if there is a substantial likelihood that an Examiner would consider it important in deciding the patentability of the invention. To the extent that such information exists, it should be submitted to the U.S. Patent and Trademark Office within three (3) months of the filing date, or as soon thereafter as possible.

Please remember that the duty to cite material prior art also extends to prior art that you may subsequently become aware of up to the time of issuance of the U.S. patent. This includes, for example, prior art cited during the prosecution of corresponding foreign applications that would be material to the examination of these applications.

Because we would like to file these documents as soon as possible, we ask that you please return the signed documents to us at your earliest convenience, and at most by August 19, 2005.

If you have any questions regarding the enclosed documents please contact me at 650-849-6614.

Very truly yours,

Lauren L. Stevens, Ph.D.

LLS/smb
Enclosures

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; I believe I am an original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: COMPOUNDS, COMPOSITIONS AND METHODS, the specification of which was filed on March 11, 2005, as United States Application No. 10/527,540.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application(s) designating at least one country other than the United States, listed below and have also identified below, any foreign application(s) for patent or inventor's certificate, or any PCT International application(s) having a filing date before that of the application(s) of which priority is claimed:

Country	Application Number	Date of Filing	Priority Claimed Under 35 U.S.C. 119
			☐ YES ☐ NO

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Number	Date of Filing
60/410,743	September 13, 2002

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application(s) and the national or PCT International filing date of this application:

Application Number	Date of Filing	Status (Patented, Pending, Abandoned)
PCT/US2003/028696	September 11, 2003	Completed

I hereby appoint the following attorney and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P., CUSTOMER NUMBER 22,852**.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of First Inventor **Han-Jie ZHOU**	Inventor's Signature	Date
Residence 280 East Grand Avenue, South San Francisco, CA 94080		Citizenship U.S.A.
Post Office Address 280 East Grand Avenue, South San Francisco, CA 94080		
Full Name of Second Inventor **Andrew McDONALD**	Inventor's Signature	Date
Residence 280 East Grand Avenue, South San Francisco, CA 94080		Citizenship China
Post Office Address 280 East Grand Avenue, South San Francisco, CA 94080		

Attorney Docket No. 09367.0044-00000

ASSIGNMENT OF PATENT APPLICATION

JOINT

WHEREAS, Han-Jie ZHOU, and Andrew McDONALD, all of 280 East Grand Avenue, South San Francisco, CA 94080; hereinafter referred to as "Assignors," are the inventors of the invention described and set forth in the below-identified application for United States Letters Patent:

Title of Invention: COMPOUNDS, COMPOSITIONS AND METHODS

International Filing Date: September 11, 2003

Application No.: 10/527,540; and

WHEREAS, Cytokietics, Inc., a corporation of Delaware, whose post office address is 280 East Grand Avenue, South San Francisco, CA 94080 (hereinafter referred to as Assignee), is desirous of securing the entire right, title, and interest in and to this invention in all countries throughout the world, and in and to the application for United States Letters Patent on this invention and the Letters Patent to be issued upon this application;

NOW THEREFORE, be it known that, for good and valuable consideration the receipt of which from Assignee is hereby acknowledged, We, as Assignors, have sold, assigned, transferred, and set over, and do hereby sell, assign, transfer, and set over unto the Assignee, its lawful successors and assigns, our entire right, title, and interest in and to this invention, Provisional Application No. 60/410,743, filed September 13, 2002, and this application, and all divisions, and continuations thereof, and all Letters Patent of the United States which may be granted thereon, and all reissues thereof, and all rights to claim priority on the basis of the above provisional application (if any), as well as all rights to claim priority on the basis of this application, and all applications for Letters Patent which may hereafter be filed for this invention in any foreign country and all Letters Patent which may be granted on this invention in any foreign country, and all extensions, renewals, and reissues thereof; and We hereby authorize and request the Commissioner of Patents and Trademarks of the United States and any official of any foreign country whose duty it is to issue patents on applications as described above, to issue all Letters Patent for this invention to Assignee, its successors and assigns, in accordance with the terms of this Assignment;

AND, WE HEREBY covenant that We have the full right to convey the interest assigned by this Assignment, and We have not executed and will not execute any agreement in conflict with this Assignment;

AND, WE HEREBY further covenant and agree that We will, without further consideration, communicate with Assignee, its successors and assigns, any facts known to us

respecting this invention, and testify in any legal proceeding, sign all lawful papers when called upon to do so, execute and deliver any and all papers that may be necessary or desirable to perfect the title to this invention in said Assignee, its successors or assigns, execute all divisional, continuation, and reissue applications, make all rightful oaths and generally do everything possible to aid Assignee, its successors and assigns, to obtain and enforce proper patent protection for this invention in the United States and any foreign country, it being understood that any expense incident to the execution of such papers shall be borne by the Assignee, its successors and assigns.

IN TESTIMONY WHEREOF, I/We have hereunto set our hands.

Han-Jie ZHOU		
Full Name of First Inventor	Inventor's Signature	Date
Andrew McDONALD		
Full Name of Second Inventor	Inventor's Signature	Date

COMPOUNDS, COMPOSITIONS AND METHODS

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application number 60/410,743 filed September 13, 2002; which is incorporated herein by reference for all purposes.

FIELD OF THE INVENTION

[0002] This invention relates to compounds which are inhibitors of the mitotic kinesin KSP and are useful in the treatment of cellular proliferative diseases, for example cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders, fungal disorders, and inflammation.

BACKGROUND OF THE INVENTION

[0003] Among the therapeutic agents used to treat cancer are the taxanes and vinca alkaloids, which act on microtubules. Microtubules are the primary structural element of the mitotic spindle. The mitotic spindle is responsible for distribution of replicate copies of the genome to each of the two daughter cells that result from cell division. It is presumed that disruption of the mitotic spindle by these drugs results in inhibition of cancer cell division, and induction of cancer cell death. However, microtubules form other types of cellular structures, including tracks for intracellular transport in nerve processes. Because these agents do not specifically target mitotic spindles, they have side effects that limit their usefulness.

[0004] Improvements in the specificity of agents used to treat cancer is of considerable interest because of the therapeutic benefits which would be realized if the side effects associated with the administration of these agents could be reduced. Traditionally, dramatic improvements in the treatment of cancer are associated with identification of therapeutic agents acting through novel mechanisms. Examples of this include not only the taxanes, but also the camptothecin class of topoisomerase I inhibitors. From both of these perspectives, mitotic kinesins are attractive targets for new anti-cancer agents.

[0005] Mitotic kinesins are enzymes essential for assembly and function of the mitotic spindle, but are not generally part of other microtubule structures, such as in

nerve processes. Mitotic kinesins play essential roles during all phases of mitosis. These enzymes are "molecular motors" that transform energy released by hydrolysis of ATP into mechanical force which drives the directional movement of cellular cargoes along microtubules. The catalytic domain sufficient for this task is a compact structure of approximately 340 amino acids. During mitosis, kinesins organize microtubules into the bipolar structure that is the mitotic spindle. Kinesins mediate movement of chromosomes along spindle microtubules, as well as structural changes in the mitotic spindle associated with specific phases of mitosis. Experimental perturbation of mitotic kinesin function causes malformation or dysfunction of the mitotic spindle, frequently resulting in cell cycle arrest and cell death.

[0006] Among the mitotic kinesins which have been identified is KSP. KSP belongs to an evolutionarily conserved kinesin subfamily of plus end-directed microtubule motors that assemble into bipolar homotetramers consisting of antiparallel homodimers. During mitosis KSP associates with microtubules of the mitotic spindle. Microinjection of antibodies directed against KSP into human cells prevents spindle pole separation during prometaphase, giving rise to monopolar spindles and causing mitotic arrest and induction of programmed cell death. KSP and related kinesins in other, non-human, organisms, bundle antiparallel microtubules and slide them relative to one another, thus forcing the two spindle poles apart. KSP may also mediate in anaphase B spindle elongation and focussing of microtubules at the spindle pole.

[0007] Human KSP (also termed HsEg5) has been described (Blangy, et al., Cell, 83:1159-69 (1995); Whitehead, et al., Arthritis Rheum., 39:1635-42 (1996); Galgio et al., J. Cell Biol., 135:339-414 (1996); Blangy, et al., J Biol. Chem., 272:19418-24 (1997); Blangy, et al., Cell Motil Cytoskeleton, 40:174-82 (1998); Whitehead and Rattner, J. Cell Sci., 111:2551-61 (1998); Kaiser, et al., JBC 274:18925-31 (1999); GenBank accession numbers: X85137, NM004523 and U37426), and a fragment of the KSP gene (TRIP5) has been described (Lee, et al., Mol Endocrinol., 9:243-54 (1995); GenBank accession number L40372). Xenopus KSP homologs (Eg5), as well as Drosophila KLP61 F/KRP1 30 have been reported.

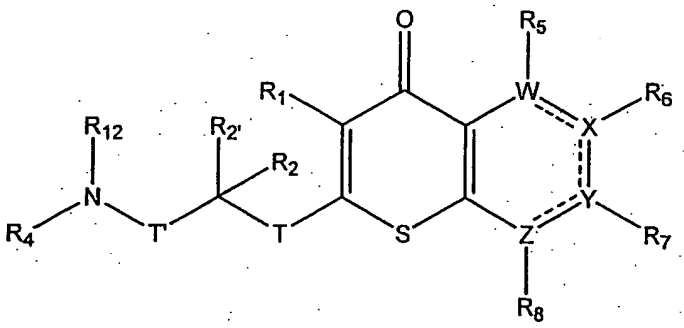
[0008] Mitotic kinesins, including KSP, are attractive targets for the discovery and development of novel antimitotic chemotherapeutics. Accordingly, it is an object of the present invention to provide compounds, compositions and methods useful in

the inhibition of KSP.

SUMMARY OF THE INVENTION

[0009] In accordance with the objects outlined above, the present invention provides compounds that can be used to treat cellular proliferative diseases. The compounds are KSP inhibitors, particularly human KSP inhibitors. The present invention also provides compositions comprising such compounds, and methods utilizing such compounds or compositions, which can be used to treat cellular proliferative diseases.

[0010] In one aspect, the invention relates to methods for treating cellular proliferative diseases, and for treating disorders by inhibiting the activity of KSP. The methods employ compounds represented by Formula I:



Formula I

wherein:

W, X, Y, and Z are independently N, C, CH, O, or S; and Z is optionally absent, provided that:

no more than two of W, X, Y, and Z is –N=, and

W, X, or Y can be O or S only when Z is absent;

the dashed lines in the structure depict optional double bonds;

T and T' are independently a covalent bond, -C(O)-, or optionally substituted lower alkylene;

R_1 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

R_2 and $R_{2'}$ are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; or R_2 and $R_{2'}$ taken together form an optionally substituted 3- to 7-membered ring;

R_{12} is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, $-C(O)-R_3$, and $-S(O)_2-R_{3a}$;

R_3 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, $R_{15}O-$ and $R_{17}-NH-$;

R_{3a} is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, and $R_{15}-NH-$;

R_4 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaralkyl-, and optionally substituted heterocyclyl-;

or R_4 taken together with R_{12}, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring;

or R_4 taken together with R_2 form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring;

R_5, R_6, R_7 and R_8 are independently chosen from hydrogen, acyl, optionally substituted alkyl-, optionally substituted alkoxy, halogen, hydroxyl, nitro, cyano, optionally substituted amino, alkylsulfonyl-, alkylsulfonamido-, alkylthio-, carboxyalkyl-, aminocarbonyl-, optionally substituted aryl and optionally substituted heteroaryl-, provided that R_5, R_6, R_7 and R_8 is absent where W, X, Y, or Z, respectively, is –N=, O, S or absent;

R_{15} is chosen from optionally substituted alkyl-, optionally substituted aryl-,

optionally substituted aralkyl-, optionally substituted heteroaryl-, and optionally substituted heteroaralkyl-; and

R_{17} is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted hetero-aralkyl-,

(Formula I including single stereoisomers and mixtures of stereoisomers);

a pharmaceutically acceptable salt of a compound of Formula I;

a pharmaceutically acceptable solvate of a compound of Formula I; or

a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I.

[0011] In one aspect, the invention relates to methods for treating cellular proliferative diseases and other disorders that can be treated by inhibiting KSP by the administration of a therapeutically effective amount of a compound of Formula I; a pharmaceutically acceptable salt of a compound of Formula I; a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I. Such diseases and disorders include cancer, hyperplasia, restenosis, cardiac hypertrophy, immune disorders, fungal disorders and inflammation.

[0012] In another aspect, the invention relates to compounds useful in inhibiting KSP kinesin. The compounds have the structures shown above in Formula I; a pharmaceutically acceptable salt of a compound of Formula I; a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I. The invention also relates to pharmaceutical compositions comprising: a therapeutically effective amount of a compound of Formula I; a pharmaceutically acceptable salt of a compound of Formula I; a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I; and one or more pharmaceutical excipients. In another aspect, the composition further comprises a chemotherapeutic agent other than a compound of the present invention.

[0013] In an additional aspect, the present invention provides methods of screening for compounds that will bind to a KSP kinesin, for example compounds that will displace or compete with the binding of a compound of the invention. The

methods comprise combining a labeled compound of the invention, a KSP kinesin, and at least one candidate agent and determining the binding of the candidate agent to the KSP kinesin.

[0014] In a further aspect, the invention provides methods of screening for modulators of KSP kinesin activity. The methods comprise combining a compound of the invention, a KSP kinesin, and at least one candidate agent and determining the effect of the candidate agent on the KSP kinesin activity.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0015] As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise. The following abbreviations and terms have the indicated meanings throughout:

Ac	=	acetyl
Boc	=	t-butyloxy carbonyl
Bu	=	butyl
c-	=	cyclo
CBZ	=	carbobenzoxy = benzyloxycarbonyl
DCM	=	dichloromethane = methylene chloride = CH_2Cl_2
DIEA	=	N,N-diisopropylethylamine
DMF	=	N,N-dimethylformamide
DMSO	=	dimethyl sulfoxide
Et	=	ethyl
Fmoc	=	9-fluorenylmethoxycarbonyl
GC	=	gas chromatography
HMDS	=	hexamethyldisilazane
HOAc	=	acetic acid
HOBt	=	hydroxybenzotriazole
Me	=	methyl
mesyl	=	methanesulfonyl
Ph	=	phenyl
PhOH	=	phenol

Py	=	pyridine
rt	=	room temperature
sat'd	=	saturated
s-	=	secondary
t-	=	tertiary
TES	=	triethylsilyl
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
TMS	=	trimethylsilyl
tosyl	=	p-toluenesulfonyl

[0016] **Alkyl** is intended to include linear, branched, or cyclic aliphatic hydrocarbon structures and combinations thereof, which structures may be saturated or unsaturated. **Lower-alkyl** refers to alkyl groups of from 1 to 5 carbon atoms, preferably from 1 to 4 carbon atoms. Examples of lower-alkyl groups include methyl-, ethyl-, propyl-, isopropyl-, butyl-, s-and t-butyl and the like. Preferred alkyl groups are those of C_{20} or below. More preferred alkyl groups are those of C_{13} or below. **Cycloalkyl** is a subset of alkyl and includes cyclic aliphatic hydrocarbon groups of from 3 to 13 carbon atoms. Examples of cycloalkyl groups include c- propyl-, c-butyl-, c-pentyl-, norbornyl-, adamantyl and the like. **Cycloalkyl-alkyl-** is another subset of alkyl and refers to cycloalkyl attached to the parent structure through a non-cyclic alkyl-. Examples of cycloalkyl-alkyl- include cyclohexylmethyl-, cyclopropylmethyl-, cyclohexylpropyl-, and the like. In this application, alkyl includes alkanyl-, alkenyl and alkynyl residues; it is intended to include vinyl-, allyl-, isoprenyl and the like. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed; thus, for example, "butyl" is meant to include n-butyl-, sec-butyl-, isobutyl and t-butyl-; "propyl" includes n-propyl-, isopropyl-, and c-propyl-.

[0017] **Alkylene-, alkenylene-, and alkynylene-** are other subsets of alkyl-, including the same residues as alkyl-, but having two points of attachment within a chemical structure. Examples of alkylene include ethylene ($-CH_2CH_2-$), propylene ($-CH_2CH_2CH_2-$), dimethylpropylene ($-CH_2C(CH_3)_2CH_2-$) and cyclohexylpropylene ($-CH_2CH_2CH(C_6H_{13})-$). Likewise, examples of alkenylene include ethenylene (-

CH=CH-), propenylene (-CH=CH-CH_2-), and cyclohexylpropenylene (-CH=CHCH(C_6H_{13})-). Examples of alkynylene include ethynylene (-C≡C-) and propynylene (-CH≡CH-CH_2-).

[0018] **Cycloalkenyl** is a subset of alkyl and includes unsaturated cyclic hydrocarbon groups of from 3 to 13 carbon atoms. Examples of cycloalkenyl groups include c-hexenyl-, c-pentenyl and the like.

[0019] **Alkoxy** or **alkoxyl** refers to an alkyl group, preferably including from 1 to 8 carbon atoms, of a straight, branched, or cyclic configuration, or a combination thereof, attached to the parent structure through an oxygen (i.e., the group alkyl-O-). Examples include methoxy-, ethoxy-, propoxy-, isopropoxy-, cyclopropyloxy-, cyclohexyloxy- and the like. **Lower-alkoxy** refers to alkoxy groups containing one to four carbons.

[0020] **Acyl** refers to groups of from 1 to 8 carbon atoms of a straight, branched, or cyclic configuration or a combination thereof, attached to the parent structure through a carbonyl functionality. Such groups may be saturated or unsaturated, and aliphatic or aromatic. One or more carbons in the acyl residue may be replaced by oxygen, nitrogen (e.g., carboxamido), or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl-, benzoyl-, propionyl-, isobutyryl-, oxalyl-, t-butoxycarbonyl-, benzyloxycarbonyl, morpholinylcarbonyl, and the like. **Lower-acyl** refers to acyl groups containing one to four carbons.

[0021] **Amino** refers to the group -NH_2. The term "substituted amino" refers to the group -NHR or -NRR where each R is independently selected from the group: optionally substituted alkyl-, optionally substituted alkoxy, optionally substituted aminocarbonyl-, optionally substituted aryl-, optionally substituted heteroaryl-, optionally substituted heterocyclyl-, acyl-, alkoxycarbonyl-, sulfanyl-, sulfinyl and sulfonyl-, e.g., diethylamino, methylsulfonylamino, furanyl-oxy-sulfonamino. Substituted amino includes the groups NR^cCOR^b, $-NR^cCO_2R^a$, and $-NR^cCONR^bR^c$, where

R^a is an optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group;

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl-; and

where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently selected from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, -OC_1-C_4 alkyl, -OC_1-C_4 alkylphenyl, -C_1-C_4 alkyl-OH, -OC_1-C_4 haloalkyl, halogen, -OH, -NH_2, -C_1-C_4 alkyl-NH_2, -N(C_1-C_4 alkyl)(C_1-C_4 alkyl), -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_4 alkylphenyl), -NH(C_1-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), -CO_2H, -C(O)OC_1-C_4 alkyl, -CON(C_1-C_4 alkyl)(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -$CONH_2$, -NHC(O)(C_1-C_4 alkyl), -NHC(O)(phenyl), -N(C_1-C_4 alkyl)C(O)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)C(O)(phenyl), -C(O)C_1-C_4 alkyl, -C(O)C_1-C_4 phenyl, -C(O)C_1-C_4 haloalkyl, -OC(O)C_1-C_4 alkyl, -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), -SO_2(C_1-C_4 haloalkyl), -SO_2NH_2, -SO_2NH(C_1-C_4 alkyl), -SO_2NH(phenyl), -NHSO_2(C_1-C_4 alkyl), -NHSO_2(phenyl), and -NHSO_2(C_1-C_4 haloalkyl).

[0022] **Antimitotic** refers to a drug for inhibiting or preventing mitosis, for example, by causing metaphase arrest. Some antitumour drugs block proliferation and are considered antimitotics.

[0023] **Aryl** and **heteroaryl** mean a 5- or 6-membered aromatic or heteroaromatic ring containing 0 or 1-4 heteroatoms, respectively, selected from O, N, or S; a bicyclic 9- or 10-membered aromatic or heteroaromatic ring system containing 0 or 1-4 (or more) heteroatoms, respectively, selected from O, N, or S; or a tricyclic 12- to 14-membered aromatic or heteroaromatic ring system containing 0 or 1-4 (or more) heteroatoms, respectively, selected from O, N, or S. The aromatic 6- to 14-membered carbocyclic rings include, e.g., phenyl-, naphthyl-, indanyl-, tetralinyl-, and fluorenyl and the 5- to 10-membered aromatic heterocyclic rings include, e.g., imidazolyl-, pyridinyl-, indolyl-, thienyl-, benzopyranonyl-, thiazolyl-, furanyl-, benzimidazolyl-, quinolinyl-, isoquinolinyl-, quinoxalinyl-, pyrimidinyl-, pyrazinyl-, tetrazolyl and pyrazolyl-.

[0024] **Aralkyl-** refers to a residue in which an aryl moiety is attached to the parent structure via an alkyl residue. Examples include benzyl-, phenethyl-, phenylvinyl-, phenylallyl and the like. **Heteroaralkyl-** refers to a residue in which a heteroaryl moiety is attached to the parent structure via an alkyl residue. Examples include furanylmethyl-, pyridinylmethyl-, pyrimidinylethyl and the like.

[0025] **Aralkoxy-** refers to the group -O-aralkyl. Similarly, **heteroaralkoxy-** refers to the group –O-heteroaralkyl-; **aryloxy-** refers to the group –O-aryl-; **acyloxy-** refers to the group –O-acyl-; **heteroaryloxy-** refers to the group –O-heteroaryl-; and **heterocyclyloxy-** refers to the group -O-heterocyclyl (i.e., aralkyl-, heteroaralkyl-, aryl-, acyl-, heterocyclyl-, or heteroaryl is attached to the parent structure through an oxygen).

[0026] **Carboxyalkyl-** refers to the group –alkyl-COOH.

[0027] **Aminocarbonyl** refers to the group $-CONR^bR^c$, where

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl-; and

where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently selected from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, -OC_1-C_4 alkyl-, -OC_1-C_4 alkylphenyl, -C_1-C_4 alkyl-OH, -OC_1-C_4 haloalkyl, halogen, -OH, $-NH_2$, -C_1-C_4 alkyl-NH_2, -N(C_1-C_4 alkyl)(C_1-C_4 alkyl), -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_4 alkylphenyl), -NH(C_1-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), $-CO_2H$, -C(O)OC_1-C_4 alkyl, -CON(C_1-C_4 alkyl)(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), $-CONH_2$, -NHC(O)(C_1-C_4 alkyl), -NHC(O)(phenyl), -N(C_1-C_4 alkyl)C(O)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)C(O)(phenyl), -C(O)C_1-C_4 alkyl, -C(O)C_1-C_4 phenyl, -C(O)C_1-C_4 haloalkyl, -OC(O)C_1-C_4 alkyl, $-SO_2$(C_1-C_4 alkyl), $-SO_2$(phenyl), $-SO_2$(C_1-C_4 haloalkyl), $-SO_2NH_2$, $-SO_2NH$(C_1-C_4 alkyl), $-SO_2NH$(phenyl), $-NHSO_2$(C_1-C_4 alkyl), $-NHSO_2$(phenyl), and $-NHSO_2$(C_1-C_4 haloalkyl). Aminocarbonyl is meant to include carbamoyl-; lower-alkyl carbamoyl-; benzylcarbamoyl-; phenylcarbamoyl-; methoxymethyl-carbamoyl-; and the like.

[0028] **Halogen** or **halo** refers to fluorine, chlorine, bromine or iodine. Fluorine, chlorine and bromine are preferred. Dihaloaryl-, dihaloalkyl-, trihaloaryl etc. refer to aryl and alkyl substituted with the designated plurality of halogens (here, 2, 2 and 3, respectively), but not necessarily a plurality of the same halogen; thus 4-chloro-3-fluorophenyl is within the scope of dihaloaryl-.

[0029] **Heterocyclyl** means a cycloalkyl or aryl residue in which one to four of the carbons is replaced by a heteroatom such as oxygen, nitrogen or sulfur. Examples

of heterocycles that fall within the scope of the invention include azetidinyl-, imidazolinyl-, pyrrolidinyl-, pyrazolyl-, pyrrolyl-, indolyl-, quinolinyl-, isoquinolinyl-, tetrahydroisoquinolinyl-, benzofuranyl-, benzodioxanyl-, benzodioxyl (commonly referred to as methylenedioxyphenyl-, when occurring as a substituent), tetrazolyl-, morpholinyl-, thiazolyl-, pyridinyl-, pyridazinyl-, piperidinyl-, pyrimidinyl-, thienyl-, furanyl-, oxazolyl-, oxazolinyl-, isoxazolyl-, dioxanyl-, tetrahydrofuranyl and the like. "N-heterocyclyl" refers to a nitrogen-containing heterocycle.

[0030] The term heterocyclyl encompasses heteroaryl-, which is a subset of heterocyclyl-. Examples of N-heterocyclyl residues include azetidinyl-, 4-morpholinyl-, 4-thiomorpholinyl-, 1-piperidinyl-, 1-pyrrolidinyl-, 3-thiazolidinyl-, piperazinyl and 4-(3,4-dihydrobenzoxazinyl). Examples of substituted heterocyclyl include 4-methyl-1-piperazinyl and 4-benzyl-1-piperidinyl-.

[0031] A **leaving group** or **atom** is any group or atom that will, under the reaction conditions, cleave from the starting material, thus promoting reaction at a specified site. Suitable examples of such groups unless otherwise specified are halogen atoms, mesyloxy, p-nitrobenzensulphonyloxy and tosyloxy groups.

[0032] **Optional** or **optionally** means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstances occurs and instances in which it does not. For example, "optionally substituted alkyl" includes "alkyl" and "substituted alkyl" as defined herein. It will be understood by those skilled in the art with respect to any group containing one or more substituents that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical and/or synthetically non-feasible and/or inherently unstable.

[0033] **Substituted alkoxy** refers to alkoxy wherein the alkyl constituent is substituted (i.e., -O-(substituted alkyl)). One suitable substituted alkoxy group is "polyalkoxy" or -O-(optionally substituted alkylene)-(optionally substituted alkoxy), and includes groups such as $-OCH_2CH_2OCH_3$, and residues of glycol ethers such as polyethyleneglycol, and $-O(CH_2CH_2O)_xCH_3$, where x is an integer of about 2-20, preferably about 2-10, and more preferably about 2-5. Another suitable substituted alkoxy group is hydroxyalkoxy or $-OCH_2(CH_2)_yOH$, where y is an integer of about 1-10, preferably about 1-4.

[0034] **Substituted-** alkyl-, aryl-, and heteroaryl- refer respectively to alkyl-,

aryl-, and heteroaryl wherein one or more (up to about 5, preferably up to about 3) hydrogen atoms are replaced by a substituent independently selected from the group: $-R^a$, $-OR^b$, $-O(C_1-C_2$ alkyl$)O-$ (e.g., methylenedioxy- or ethylenedioxy-), $-SR^b$, guanidine, guanidine wherein one or more of the guanidine hydrogens are replaced with a lower-alkyl group, $-NR^bR^c$, halogen, cyano, nitro, $-COR^b$, $-CO_2R^b$, $-CONR^bR^c$, $-OCOR^b$, $-OCO_2R^a$, $-OCONR^bR^c$, $-NR^cCOR^b$, $-NR^cCO_2R^a$, $-NR^cCONR^bR^c$, $-CO_2R^b$, $-CONR^bR^c$, $-NR^cCOR^b$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^bR^c$, and $-NR^cSO_2R^a$,

where R^a is an optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group,

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group;

R^c is hydrogen or C_1-C_4 alkyl-;

where each optionally substituted R^a group and R^b group is independently unsubstituted or substituted with one or more substituents independently selected from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, $-OC_1-C_4$ alkyl-, $-OC_1-C_4$ alkylphenyl-, $-C_1-C_4$ alkyl-OH, $-OC_1-C_4$ haloalkyl-, halogen, -OH, $-NH_2$, $-C_1-C_4$ alkyl-NH_2, $-N(C_1-C_4$ alkyl$)(C_1-C_4$ alkyl$)$, $-NH(C_1-C_4$ alkyl$)$, $-N(C_1-C_4$ alkyl$)(C_1-C_4$ alkylphenyl$)$, $-NH(C_1-C_4$ alkylphenyl$)$, cyano, nitro, oxo (as a substitutent for heteroaryl), $-CO_2H$, $-C(O)OC_1-C_4$ alkyl-, $-CON(C_1-C_4$ alkyl$)(C_1-C_4$ alkyl$)$, $-CONH(C_1-C_4$ alkyl$)$, $-CONH_2$, $-NHC(O)(C_1-C_4$ alkyl$)$, -NHC(O)(phenyl), $-N(C_1-C_4$ alkyl$)C(O)(C_1-C_4$ alkyl$)$, $-N(C_1-C_4$ alkyl$)C(O)$(phenyl), $-C(O)C_1-C_4$ alkyl-, $-C(O)C_1-C_4$ phenyl-, $-C(O)C_1-C_4$ haloalkyl-, $-OC(O)C_1-C_4$ alkyl-, $-SO_2(C_1-C_4$ alkyl$)$, $-SO_2$(phenyl), $-SO_2(C_1-C_4$ haloalkyl$)$, $-SO_2NH_2$, $-SO_2NH(C_1-C_4$ alkyl$)$, $-SO_2NH$(phenyl), $-NHSO_2(C_1-C_4$ alkyl$)$, $-NHSO_2$(phenyl), and $-NHSO_2(C_1-C_4$ haloalkyl$)$. In the compounds of Formula I where T and/or T' are substituted alkylene, the term "substituted" also refers to alkylene groups where one or more (up to about 3, particularly 1) carbon atoms are replaced by a heteroatom independently selected from O, N or S, such as $-CH_2-S-CH_2-$.

[0035] **Sulfanyl** refers to the groups: -S-(optionally substituted alkyl), -S-(optionally substituted aryl), -S-(optionally substituted heteroaryl), and -S-(optionally substituted heterocyclyl).

[0036] **Sulfinyl** refers to the groups: -S(O)-H, -S(O)-(optionally substituted

alkyl), -S(O)-optionally substituted aryl), -S(O)-(optionally substituted heteroaryl), -S(O)-(optionally substituted heterocyclyl); and -S(O)-(optionally substituted amino).

[0037] **Sulfonyl** refers to the groups: $-S(O_2)-H$, $-S(O_2)-$(optionally substituted alkyl), $-S(O_2)-$optionally substituted aryl), $-S(O_2)-$(optionally substituted heteroaryl), $-S(O_2)-$(optionally substituted heterocyclyl) ,$-S(O_2)-$(optionally substituted alkoxy), $-S(O_2)-$optionally substituted aryloxy), $-S(O_2)-$(optionally substituted heteroaryloxy), $-S(O_2)-$(optionally substituted heterocyclyloxy); and $-S(O_2)-$(optionally substituted amino).

[0038] **Pharmaceutically acceptable salts** refers to those salts that retain the biological effectiveness of the free compound and that are not biologically undesirable or unsuitable for pharmaceutical use, formed with a suitable acid or base, and includes pharmaceutically acceptable acid addition salts and base addition salts.

Pharmaceutically acceptable acid addition salts include those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and those derived from organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0039] **Pharmaceutically acceptable base addition salts** include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particular embodiments are the ammonium, potassium, sodium, calcium, and magnesium salts. Base addition salts also include those derived from pharmaceutically acceptable organic non-toxic bases, including salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[0040] **Protecting group** has the meaning conventionally associated with it in organic synthesis, i.e. a group that selectively blocks one or more reactive sites in a multifunctional compound such that a chemical reaction can be carried out selectively on another unprotected reactive site and such that the group can readily be removed

after the selective reaction is complete. A variety of protecting groups are disclosed, for example, in T.H. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, Third Edition, John Wiley & Sons, New York (1999), which is incorporated herein by reference in its entirety. For example, a hydroxy protected form is where at least one of the hydroxyl groups present in a compound is protected with a hydroxy protecting group. Likewise, amines and other reactive groups may similarly be protected.

[0041] **Solvate** refers to the compound formed by the interaction of a solvent and a compound of Formula I or salt thereof. Suitable solvates of the compounds of the Formula I or a salt thereof are pharmaceutically acceptable solvates including hydrates.

[0042] Many of the compounds described herein contain one or more asymmetric centers (e.g. the carbon to which R_2 and $R_{2'}$ are attached where R_2 differs from $R_{2'}$) and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)-. The present invention is meant to include all such possible isomers, including racemic mixtures, optically pure forms and intermediate mixtures. Optically active (R)- and (S)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms and rotational isomers are also intended to be included.

[0043] When desired, the R- and S-isomers may be resolved by methods known to those skilled in the art, for example by formation of diastereoisomeric salts or complexes which may be separated, for example, by crystallization; via formation of diastereoisomeric derivatives which may be separated, for example, by crystallization, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic oxidation or reduction, followed by separation of the modified and unmodified enantiomers; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support, such as silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another

chemical entity by one of the separation procedures described above, a further step may be required to liberate the desired enantiomeric form. Alternatively, specific enantiomer may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer to the other by asymmetric transformation.

Compounds of the Present Invention

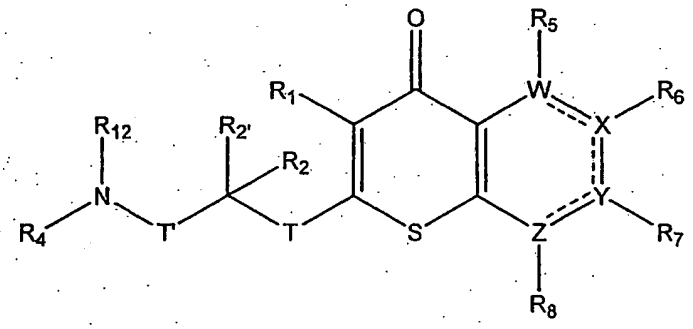
[0044] The present invention is directed to a class of novel compounds, that can be described as thiochromenone derivatives, that are inhibitors of one or more mitotic kinesins. By inhibiting mitotic kinesins, but not other kinesins (e.g., transport kinesins), specific inhibition of cellular proliferation is accomplished. While not intending to be bound by any theory, the present invention capitalizes on the finding that perturbation of mitotic kinesin function causes malformation or dysfunction of mitotic spindles, frequently resulting in cell cycle arrest and cell death. According to one embodiment of the invention, the compounds described herein inhibit the mitotic kinesin, KSP, particularly human KSP. In another embodiment, the compounds inhibit the mitotic kinesin, KSP, as well as modulating one or more of the human mitotic kinesins selected from the group consisting of HSET (see, U.S. Patent No. 6,361,993, which is incorporated herein by reference); MCAK (see, U.S. Patent No. 6,331,424, which is incorporated herein by reference); CENP-E (see, PCT Publication No. WO 99/13061 , which is incorporated herein by reference); Kif4 (see, U.S. Patent No. 6,440,684, which is incorporated herein by reference); MKLP1 (see, U.S. Patent No. 6,448,025, which is incorporated herein by reference); Kif15 (see, U.S. Patent No. 6,355,466, which is incorporated herein by reference); Kid (see, U.S. Patent No. 6,387,644, which is incorporated herein by reference); Mpp1, CMKrp, KinI-3 (see, U.S. Patent No. 6,461,855, which is incorporated herein by reference); Kip3a (see, PCT Publication No. WO 01/96593, which is incorporated herein by reference); Kip3d (see, U.S. Patent No. 6,492,151, which is incorporated herein by reference); and RabK6.

[0045] The methods of inhibiting a mitotic kinesin comprise contacting an inhibitor of the invention with a kinesin, particularly a human kinesin, more particularly, human KSP or fragments and variants thereof. The inhibition can be of the ATP hydrolysis activity of the KSP kinesin and/or the mitotic spindle formation

activity, such that the mitotic spindles are disrupted. Meiotic spindles may also be disrupted.

[0046] The present invention provides inhibitors of mitotic kinesins, in particular KSP and especially human KSP, for the treatment of disorders associated with cell proliferation. The compounds, compositions and methods described herein can differ in their selectivity and are used to treat diseases of cellular proliferation, including, but not limited to cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders, fungal disorders and inflammation.

[0047] Accordingly, the present invention relates to methods employing compounds represented by Formula I:



Formula I

wherein

W, X, Y, and Z are independently N, C, CH, O, or S; and Z is optionally absent, provided that:

no more than two of W, X, Y, and Z is –N=, and

W, X, or Y can be O or S only when Z is absent;

the dashed lines in the structure depict optional double bonds;

T and T' are independently a covalent bond, -C(O)-, or optionally substituted lower alkylene;

R_1 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

R_2 and $R_{2'}$ are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; or R_2 and $R_{2'}$ taken together form an optionally substituted 3- to 7-membered ring;

R_{12} is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, $-C(O)-R_3$, and $-S(O)_2-R_{3a}$;

R_3 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, $R_{15}O-$ and $R_{17}-NH-$;

R_{3a} is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, and $R_{15}-NH-$;

R_4 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaralkyl-, and optionally substituted heterocyclyl-;

or R_4 taken together with R_{12}, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring;

or R_4 taken together with R_2 form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring;

R_5, R_6, R_7 and R_8 are independently chosen from hydrogen, acyl, optionally substituted alkyl-, optionally substituted alkoxy, halogen, hydroxyl, nitro, cyano, optionally substituted amino, alkylsulfonyl-, alkylsulfonamido-, alkylthio-, carboxyalkyl-, aminocarbonyl-, optionally substituted aryl and optionally substituted heteroaryl-, provided that R_5, R_6, R_7 and R_8 is absent where W, X, Y, or Z, respectively, is –N=, O, S or absent;

R_{15} is chosen from optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, and optionally substituted heteroaralkyl-; and

R_{17} is hydrogen, optionally substituted alkyl-, optionally substituted aryl-,

optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted hetero-aralkyl-,

including single stereoisomers and mixtures of stereoisomers;

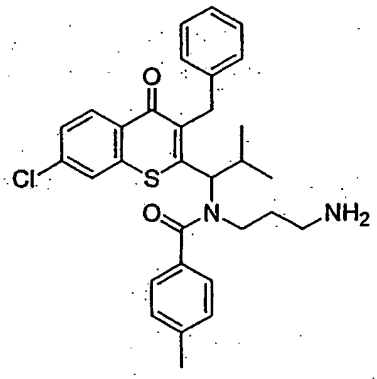
a pharmaceutically acceptable salt of a compound of Formula I;

a pharmaceutically acceptable solvate of a compound of Formula I;

or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I. In a particular embodiment, the stereogenic center to which R_2 and $R_{2'}$ are attached is of the R configuration.

Nomenclature

[0048] The compounds of Formula I can be named and numbered in the manner (e.g., using AutoNom version 2.1 in ISIS-DRAW or ChemDraw) described below. For example, the compound:



i.e., the compound according to Formula I where W, X, Y, and Z are C, T and T' are absent, R_1 is benzyl, R_2 is propyl (specifically, i -propyl), $R_{2'}$ is hydrogen; R_{12} is $-C(O)R_3$; R_3 is p-tolyl; R_4 is 3-aminopropyl; R_5, R_6, and R_8 are hydrogen; and R_7 is chloro can be named N-(3-amino-propyl)-N-[1-(3-benzyl-7-chloro-4-oxo-4H-thiochromen-2-yl)-2-methyl-propyl]-4-methyl-benzamide.

Synthetic Reaction Parameters

[0049] The compounds of Formula I can be prepared by following the procedures described with reference to the Reaction Schemes below.

[0050] Unless specified otherwise, the terms "solvent", "inert organic solvent"

or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform, methylene chloride (or dichloromethane), diethyl ether, methanol, pyridine and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert organic solvents.

[0051] The term "q.s." means adding a quantity sufficient to achieve a stated function, e.g., to bring a solution to the desired volume (i.e., 100%).

[0052] In general, esters of carboxylic acids may be prepared by conventional esterification procedures, for example alkyl esters may be prepared by treating the required activated carboxylic acid with the appropriate alkanol, generally under acidic conditions. Likewise, amides may be prepared using conventional amidation procedures, for example amides may be prepared by treating an activated carboxylic acid with the appropriate amine. Alternatively, a lower-alkyl ester such as a methyl ester of the acid may be treated with an amine to provide the required amide, optionally in presence of trimethylalluminium following the procedure described in Tetrahedron Lett. 48, 4171-4173, (1977). Carboxyl groups may be protected as alkyl esters, for example methyl esters, which esters may be prepared and removed using conventional procedures, one convenient method for converting carbomethoxy to carboxyl is to use aqueous lithium hydroxide.

[0053] The salts and solvates of the compounds mentioned herein may as required be produced by methods conventional in the art. For example, if an inventive compound is an acid, a desired base addition salt can be prepared by treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary, or tertiary); an alkali metal or alkaline earth metal hydroxide; or the like. Illustrative examples of suitable salts include organic salts derived from amino acids such as glycine and arginine; ammonia; primary, secondary, and tertiary amines; such as ethylenediamine, and cyclic amines, such as cyclohexylamine, piperidine, morpholine, and piperazine; as well as inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum, and lithium.

[0054] If a compound is a base, a desired acid addition salt may be prepared by any suitable method known in the art, including treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid,

phosphoric acid, and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosidyl acid, such as glucuronic acid or galacturonic acid, alpha-hydroxy acid, such as citric acid or tartaric acid, amino acid, such as aspartic acid or glutamic acid, aromatic acid, such as benzoic acid or cinnamic acid, sulfonic acid, such as p-toluenesulfonic acid, methanesulfonic acid, ethanesulfonic acid, or the like.

[0055] Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

Synthesis of the Compounds of Formula I

[0056] The compounds of Formula I can be prepared by following the procedures described with reference to the Reaction Schemes below.

Brief Description Of Reaction Schemes

[0057] Reaction Scheme 1 illustrates a synthesis of compounds of formula 113, an intermediate in the synthesis of compounds of Formula I.

[0058] Reaction Scheme 2 illustrates a synthesis of compounds of Formula I wherein R_{12} is $-C(O)R_3$.

[0059] Reaction Scheme 3 illustrates a synthesis of compounds of Formula I wherein R_7 is $-OH$.

[0060] Reaction Scheme 4 illustrates a synthesis of compounds of Formula I wherein R_7 is $-OCH_3$.

[0061] Reaction Scheme 5 illustrates a synthesis of compounds of Formula I wherein R_{12} is $-SO_2R_{3a}$.

[0062] Reaction Scheme 6 illustrates a synthesis of compounds of Formula I.

[0063] Reaction Scheme 7 illustrates a synthesis of compounds of Formula I

wherein R_{12} together with R_4 and the nitrogen to which they are bound, form an optionally substituted imidazolyl.

[0064] Reaction Scheme 8 illustrates another synthesis of compounds of Formula I wherein R_{12} together with R_4 and the nitrogen to which they are bound, form an optionally substituted imidazolyl.

[0065] Reaction Scheme 9 illustrates a synthesis of compounds of Formula I wherein R_{12} together with R_4 and the nitrogen to which they are bound, form an optionally substituted imidazolinyl.

[0066] Reaction Scheme 10 illustrates a second synthesis of compounds of Formula I wherein R_{12} together with R_4 and the nitrogen to which they are bound, form an optionally substituted imidazolinyl.

[0067] Reaction Scheme 11 illustrates a synthesis of compounds of Formula I wherein R_{12} is $-C(O)R_3$ wherein R_3 is $-OR_{15}$.

[0068] Reaction Scheme 12 illustrates a synthesis of compounds of Formula I wherein R_{12} is $-C(O)R_3$ wherein R_3 is $-NHR_{17}$.

[0069] Reaction Scheme 13 illustrates a synthesis of compounds of Formula I wherein R_{12} together with R_4 and the nitrogen to which they are bound, form an optionally substituted diazepinone.

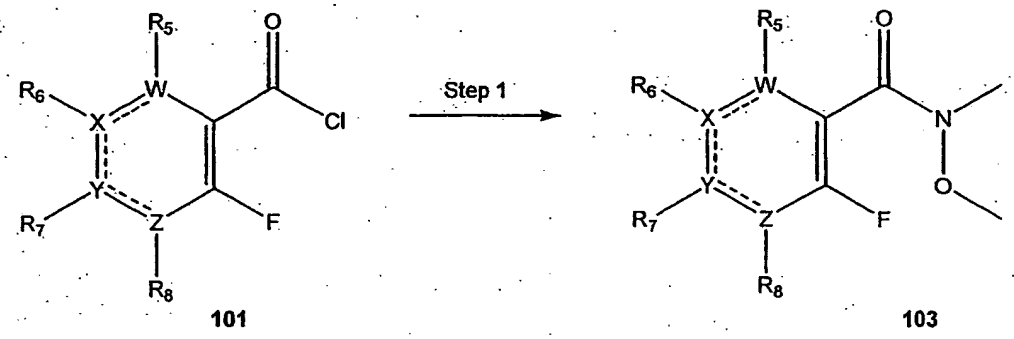
[0070] Reaction Scheme 14 illustrates a synthesis of compounds of Formula I wherein R_{12} together with R_4 and the nitrogen to which they are bound, form an optionally substituted diazepinone.

[0071] Reaction Scheme 15 illustrates a synthesis of compounds of Formula I wherein R_{12} together with R_4 and the nitrogen to which they are bound, form an optionally substituted piperazine ring.

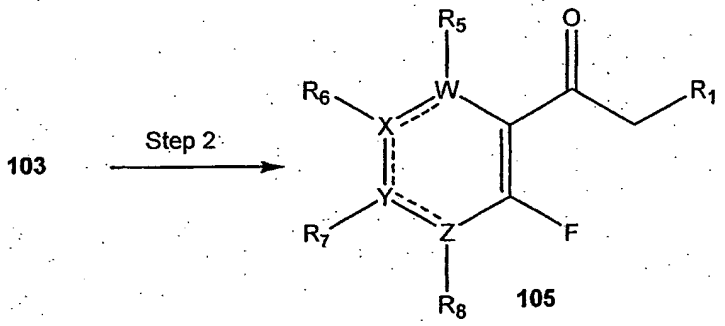
Starting Materials

[0072] The optionally substituted compounds of Formula 101 are commercially available, e.g., from Aldrich Chemical Company, Milwaukee, WI. Other reactants are likewise commercially available or may be readily prepared by those skilled in the art using commonly employed synthetic methodology. See, also, PCT WO 03/39460, WO 03/49678, WO 03/50122, WO 03/49527, WO 0349679, WO 03/50064, each of which is incorporated herein by reference for all purposes.

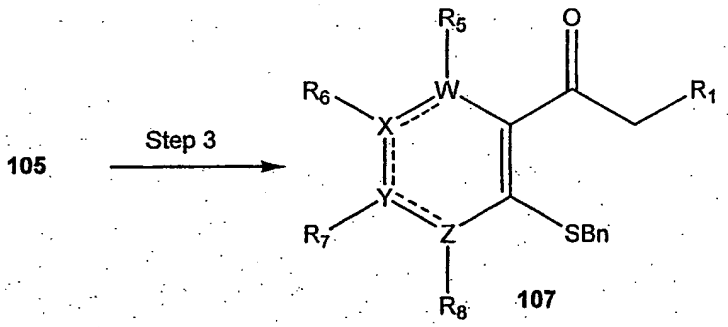
Reaction Scheme 1



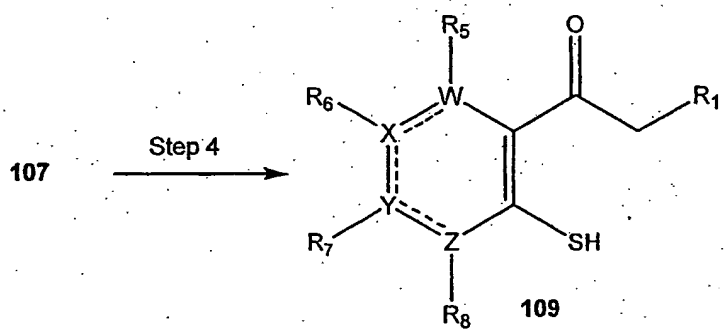
101 103



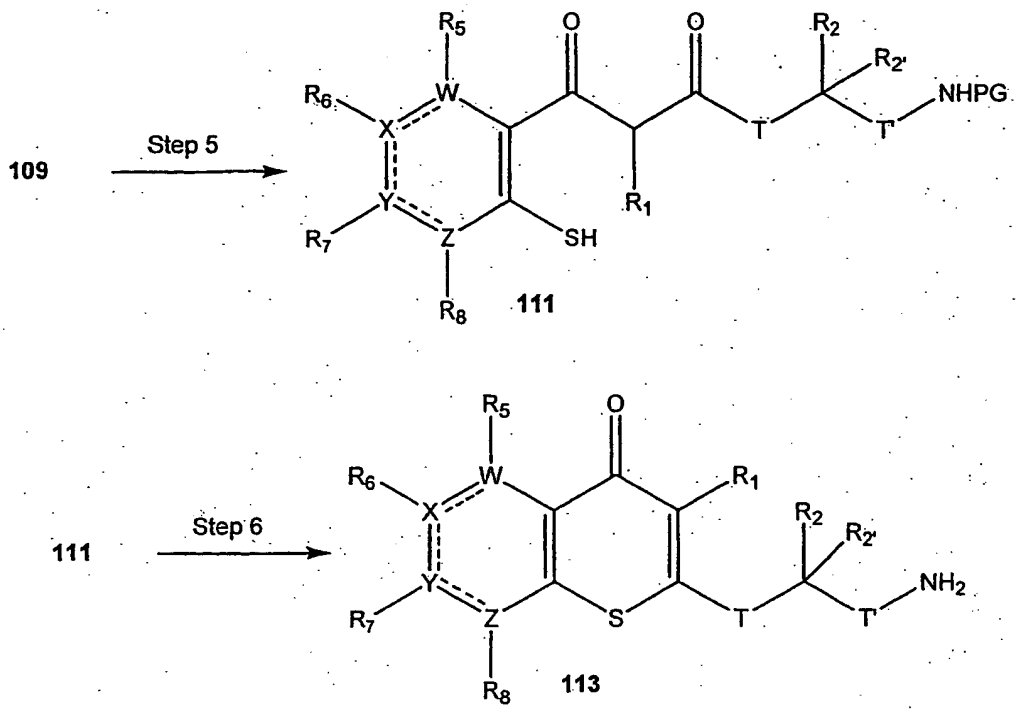
105



107



109



Preparation of Compounds of Formula 103

[0073] Referring to Reaction Scheme 1, Step 1, *N,N*-dimethylhydroxylamine hydrochloride (preferably about 1.2 equivalents) is added to a 0 °C solution of a compound of Formula 101 and a base such as triethylamine in a nonpolar, aprotic solvent such as CH_2Cl_2. After about 15 minutes, the product, a compound of Formula 103, is isolated and used without further purification.

Preparation of Compounds of Formula 105

[0074] Referring to Reaction Scheme 1, Step 2, a compound of the formula R_1CH_2Br (about 1.2 equivalents), magnesium turnings and a nonpolar, aprotic solvent such as THF are mixed at about room temperature under an inert atmosphere. After ~10 minutes the reaction mixture begins to exotherm and the reaction mixture is allowed to progress to reflux. After 1.5 hour, the Grignard reaction is complete and the solution is cooled to room temperature.

[0075] A solution of a compound of Formula 103 and a nonpolar, aprotic solvent, such as THF, is added via cannula to the solution of the Grignard reagent

prepared above. The temperature is monitored by internal thermometer and was not allowed to exceed ~40°C. After about 3 hours at room temperature, the product, a compound of Formula 105, is isolated and purified.

Preparation of Compounds of Formula 107

[0076] Referring to Reaction Scheme 1, Step 3, a base, such as sodium hydride is added to a room temperature solution of about an equivalent of α-toluenethiol in a polar, aprotic solvent such as DMF. After about 30 minutes, a compound of Formula 105 is added in one portion. After about 1 hour at room temperature, the product, a compound of Formula 107, is isolated and used without further purification.

Preparation of Compounds of Formula 109

[0077] Referring to Reaction Scheme 1, Step 4, aluminum trichloride (about 2.75 equivalents) is added to a room temperature solution of a compound of formula 107 in a nonpolar, aprotic solvent such as toluene. After about one hour at room temperature, the product, a compound of Formula 109, is isolated and purified.

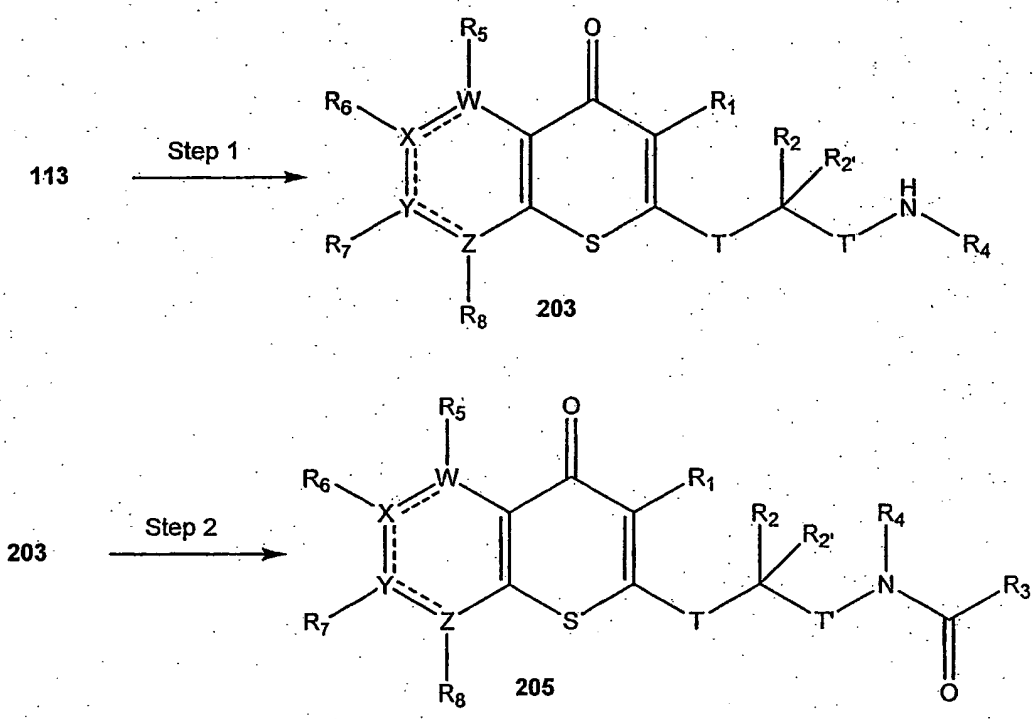
Preparation of Compounds of Formula 111

[0078] Referring to Reaction Scheme 1, Step 5, a strong base, such as lithium bis(trimethylsilyl)amide (preferably about 1.0 M in THF and more preferably more than about 4 equivalents) is added to a –78°C solution of a compound of Formula 109 in a nonpolar, aprotic solvent such as THF. After the addition was complete the resulting solution is maintained at –78°C for about 1 hour. The reaction solution is then warmed to 0°C for about 2 hours. The reaction solution is then cooled to –78°C. A solution of acid fluoride of the Formula F-(CO)-R-C(R_2)($R_{2'}$)-T'-(NHPG) and a nonpolar, aprotic solvent such as THF is added drop-wise via syringe to the reaction solution. After about 15 minutes at –78°C, the reaction solution is warmed to room temperature. After about 2 hours, the product, a compound of Formula 111, is isolated and used without further purification.

Preparation of Compounds of Formula 113

[0079] Referring to Reaction Scheme 1, Step 6, a solution of a compound of Formula 111 and 30% HBr in AcOH is maintained at room temperature for about one hour. The product, a compound oF Formula 113, is isolated and purified.

Reaction Scheme 2



Preparation of Formula 203

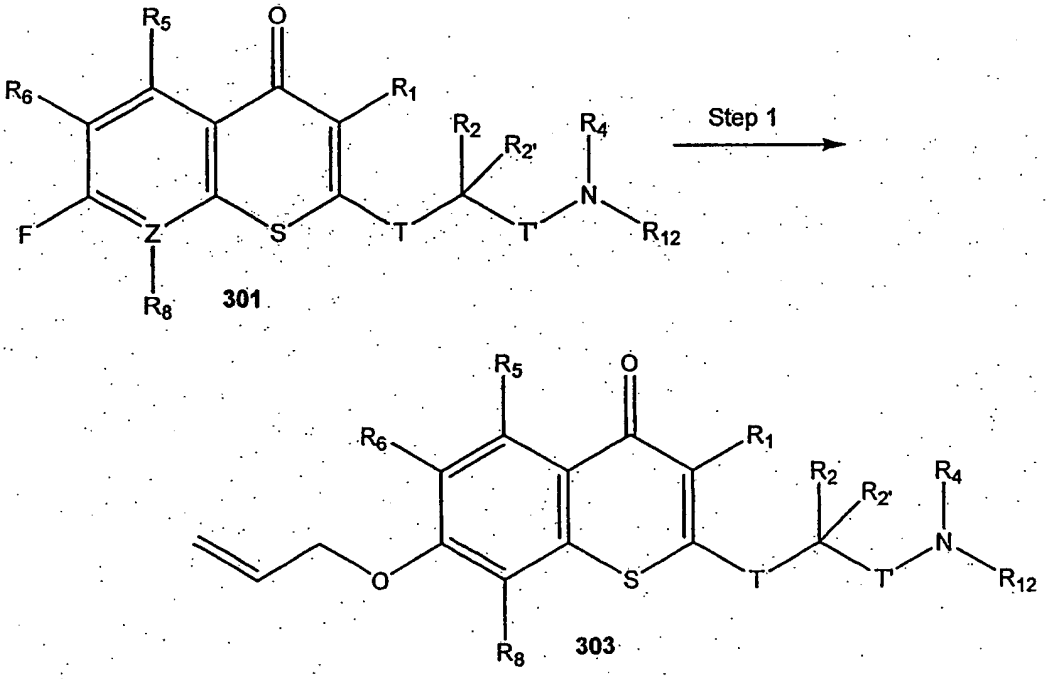
[0080] Referring to Reaction Scheme 2, Step 1, to a solution of a compound of Formula 113 is added successively a slight excess (preferably about 1.2 equivalents) of an aldehyde comprising R_4 (i.e., a compound having the formula R_4-CHO where R_4-CH_2- is equivalent to R_4 and R_4 is as described above or is a protected precursor to such a substituent, e.g., (3-oxo-propyl)-carbamic acid *tert*-butyl ester) and a reducing agent such as sodium triacetoxyborohydride. The resulting mixture is stirred for several hours. The product, a compound of Formula 203 is isolated and purified.

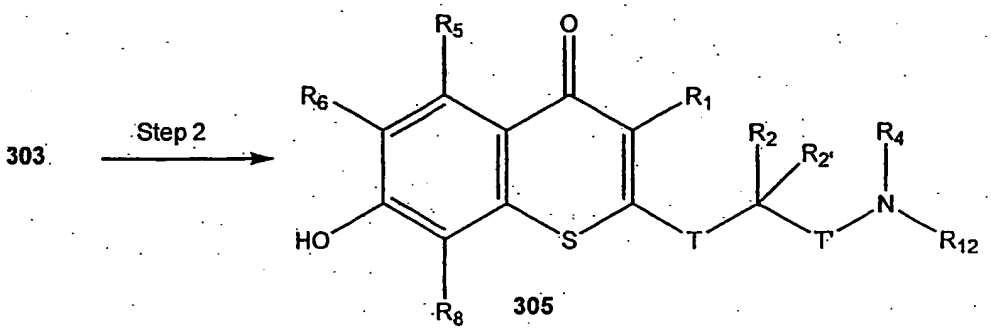
Preparation of Formula 205

[0081] Referring to Reaction Scheme 2, Step 2, to a solution of a compound of Formula 203 and an amine base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane is added an R_3 acyl chloride (such as Cl-C(O)-R_3 where R_3 is as described above). The resulting solution is stirred under nitrogen at room temperature for several hours. The product, a compound of Formula 205 is isolated and purified.

[0082] Optionally, any protecting groups on compounds of Formula 205 are then removed. For example, if R_4 comprises a protected amine wherein the protecting group is a Boc group, the Boc group can be removed by treatment of the compound of Formula 205 with an acid such as trifluoroacetic acid in a nonpolar, aprotic solvent such as dichloromethane, while maintaining the reaction at about room temperature. The reaction is monitored e.g., by TLC. Upon completion, the product, the free amine, is isolated and purified.

Reaction Scheme 3





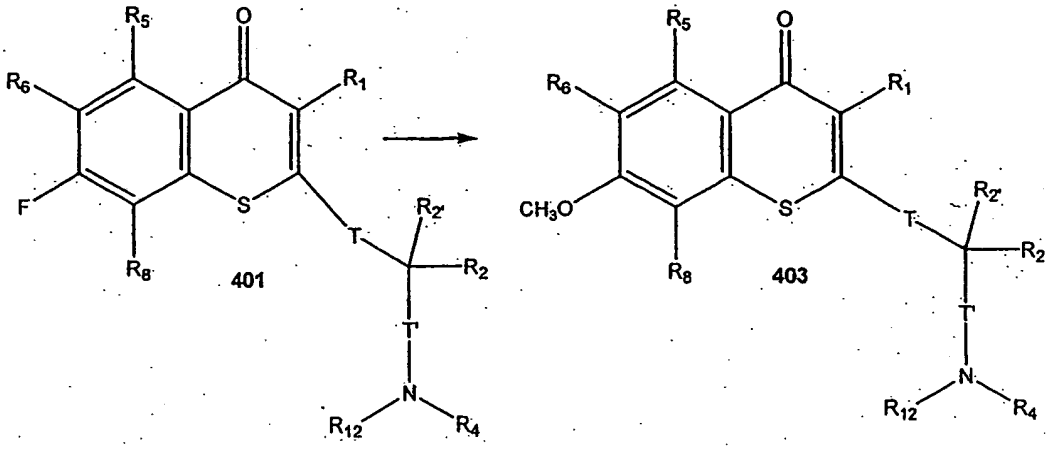
Preparation of Compounds of Formula 303

[0083] Referring to Reaction Scheme 3, Step 1, to a solution of a compound of Formula 301 in a nonpolar, aprotic solvent such as DMF is added NaH. The resulting solution was stirred at 45°C for about 5 minutes, then allyl alcohol (about 1.4 equivalents) is added via pipette. The resulting solution is stirred at 45°C for about 12 hours and then cooled to room temperature. The product, a compound of Formula 303, is isolated and used without further purification.

Preparation of Compounds of Formula 305

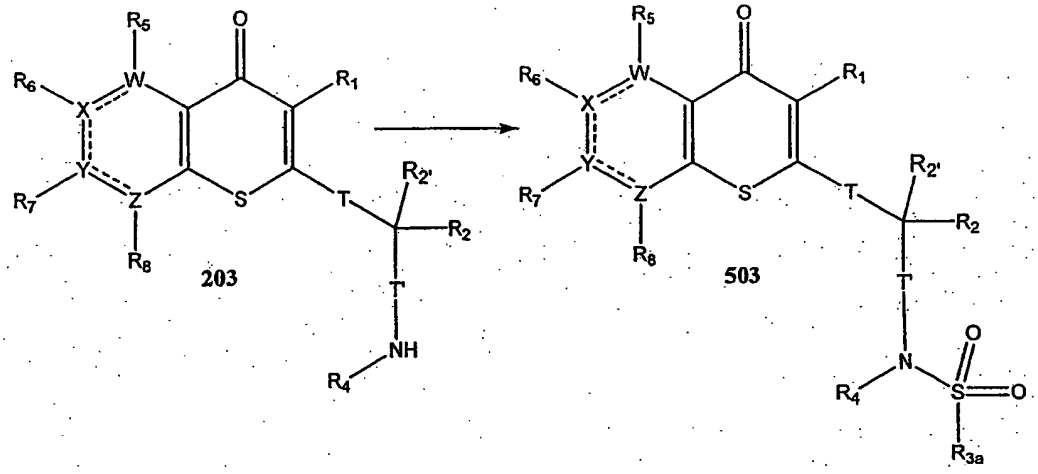
[0084] Referring to Reaction Scheme 3, Step 2, to a room temperature solution of a compound of Formula 303, in an aprotic solvent such as acetonitrile, is added morpholine followed by $Pd(PPh_3)_4$. The resulting solution is stirred for 5 about minutes. The product, a compound of Formula 305, is isolated and purified.

Reaction Scheme 4



[0085] Referring to Reaction Scheme 4, a compound of Formula 401 is dissolved in 0.5 M sodium methoxide in methanol and heated to about 70°C. The temperature is maintained at about 70°C for about 12 hours and then cooled to room temperature. The product, a compound of Formula 403, is isolated and purified.

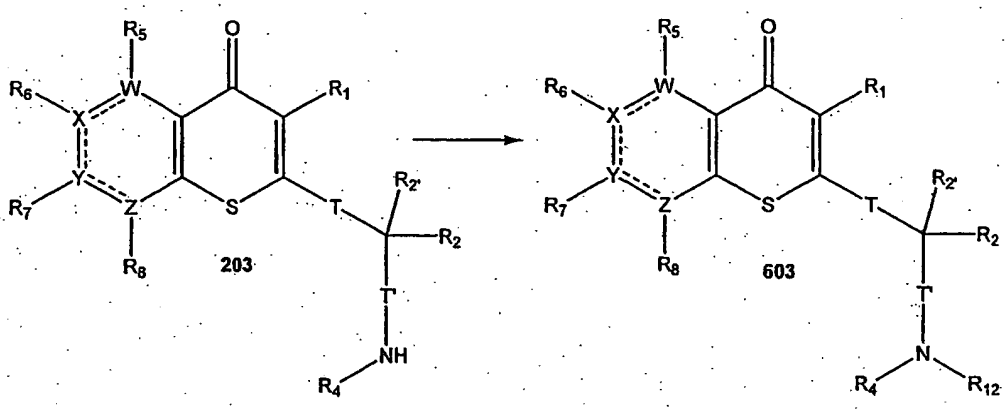
Reaction Scheme 5



[0086] Referring to Reaction Scheme 5, to a solution of a compound of Formula 203 and an amine base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane is added a compound having the formula

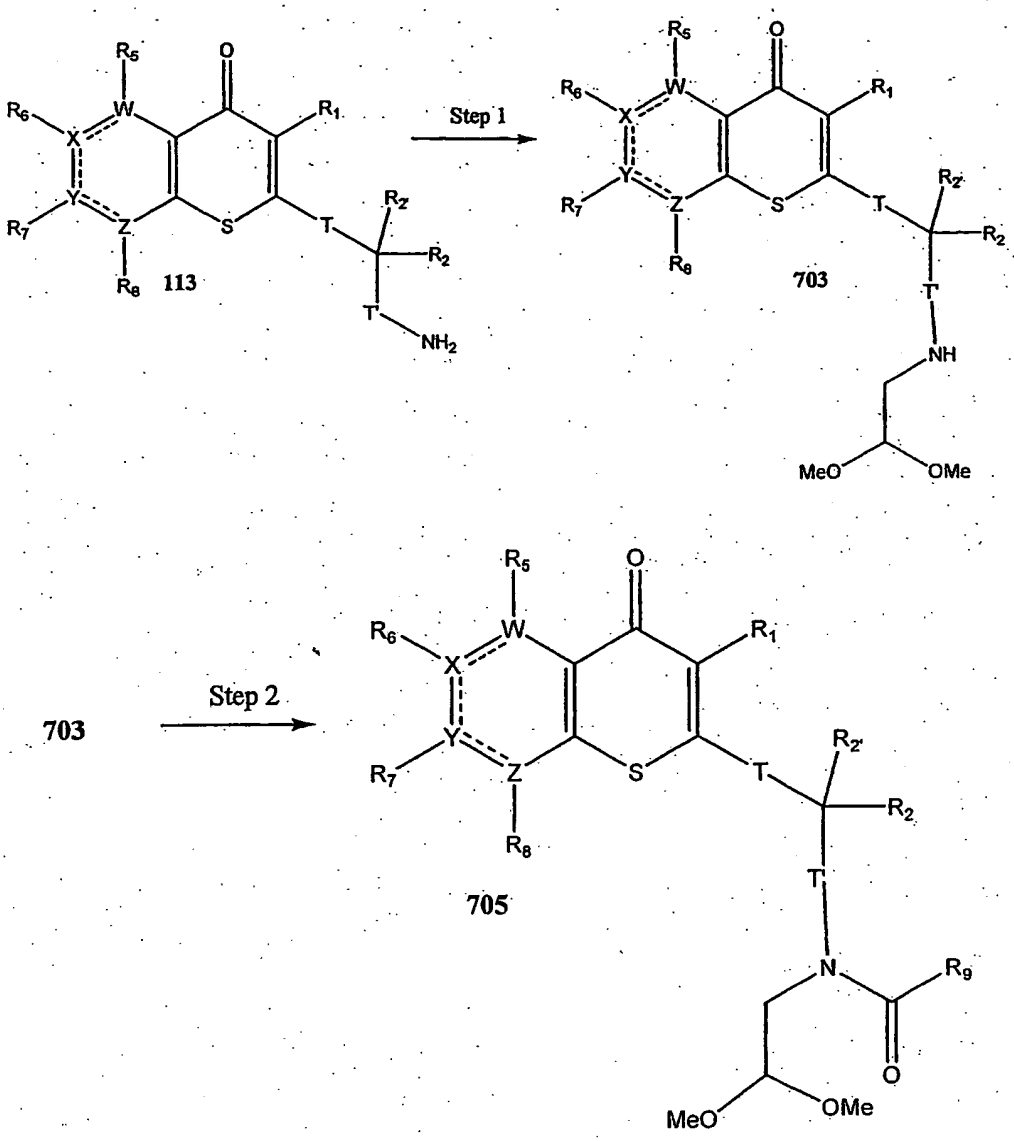
Cl-$S(O)_2$-R_{3a} or O-$(S(O)_2$-$R_{3a})_2$ where R_{3a} is as described above. The resulting solution is stirred under nitrogen at room temperature for several hours. The product, a compound of Formula 503 is isolated and purified.

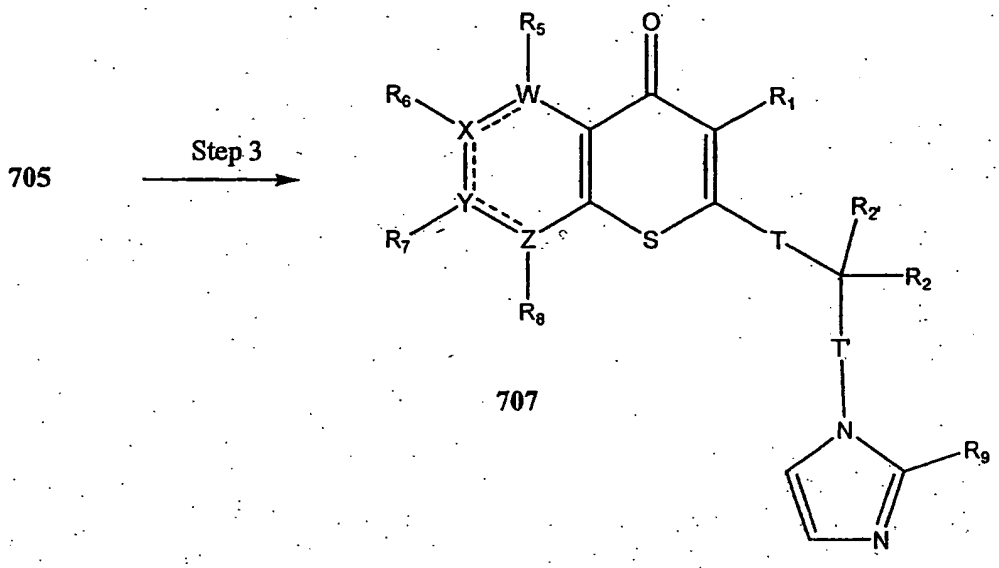
Reaction Scheme 6



[0087] Referring to Reaction Scheme 6, to a solution of a compound of Formula 203 and an amine base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane is added a compound having the formula X- R_{12} where R_{12} is as described above and X is Br or Cl. The resulting solution is stirred under nitrogen at room temperature or with heat for several hours. The product, a compound of Formula 603 is isolated and purified.

Reaction Scheme 7


Step 1
113
703
Step 2
703
705



707

Preparation of Formula 703

[0088] Referring to Reaction Scheme 7, Step 1, to an optionally substituted compound of Formula 113 dissolved in a polar, aprotic solvent (such as DMF) in the presence of a base (such as potassium carbonate) is added one equivalent of an optionally substituted suitably protected aldehyde wherein such aldehyde further comprises a leaving group, preferably, a halide. The solution is heated at reflux, monitoring completion of the reaction (e.g., by TLC). The reaction mixture is cooled and the corresponding, optionally substituted compound of Formula 703 is isolated and purified.

Preparation of Formula 705

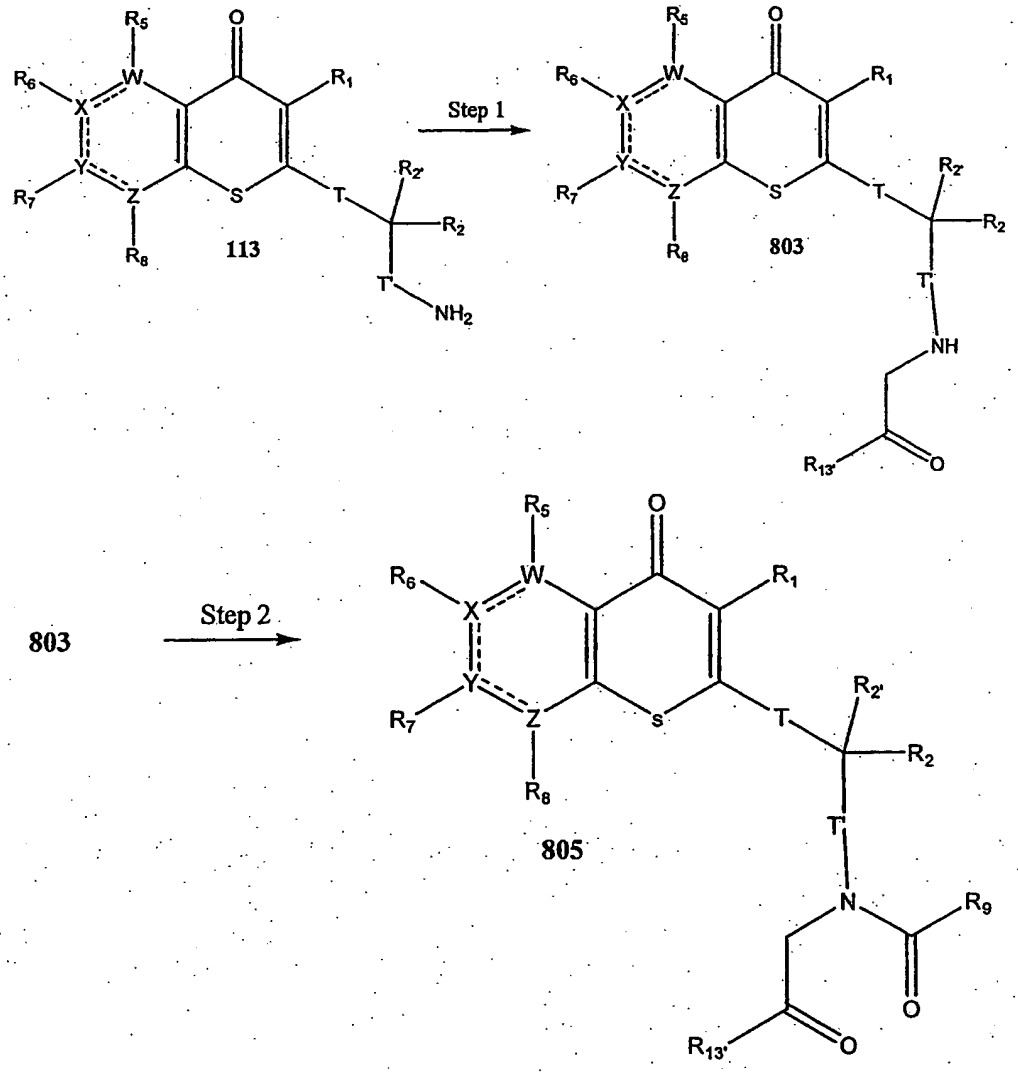
[0089] Referring to Reaction Scheme 7, Step 2, to an optionally substituted compound of Formula 703 in an inert solvent (such as dichloromethane) in the presence of about 1.5 molar equivalents of an amine base (such as triethylamine) is added about 1.5 molar equivalents of an R_9 acid chloride, such as, Cl-C(O)-R_9, where R_9 is as described above. The reaction takes place, with stirring, at room temperature over a period of 4 to 24 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 705 is isolated and purified.

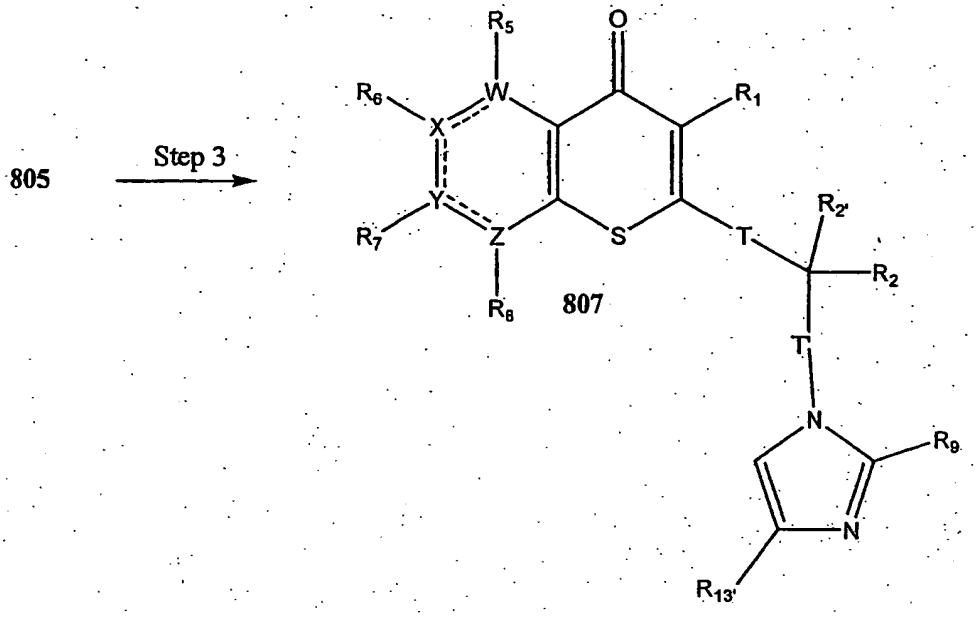
Preparation of Formula 707

[0090] Referring to Reaction Scheme 7, Step 3, a solution of a compound of

Formula 805 and an excess of ammonium acetate in acetic acid is heated at reflux for 1-4 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 707 is isolated and purified.

Reaction Scheme 8





Preparation of Formula 803

[0091] Referring to Reaction Scheme 8, Step 1, a suspension of a compound of Formula 113, an alpha-haloketone reagent of the Formula $R_{13'}(CO)CH_2X$ wherein X is a halide and $R_{13'}$ is as described herein, and about an equivalent of a base, such as potassium carbonate in a polar, aprotic solvent such as DMF is stirred at room temperature. The reaction is diluted with water and the resulting solid, a compound of Formula 803, is used in the subsequent step without further purification.

Preparation of Formula 805

[0092] Referring to Reaction Scheme 8, Step 2, a solution of the compound of Formula 803, about an equivalent of an amine base, such as triethylamine and about an equivalent of an acid chloride (such as a compound of Formula R_9-COCl) in an organic solvent such as methylene chloride is stirred at room temperature for several hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 805 is isolated and purified.

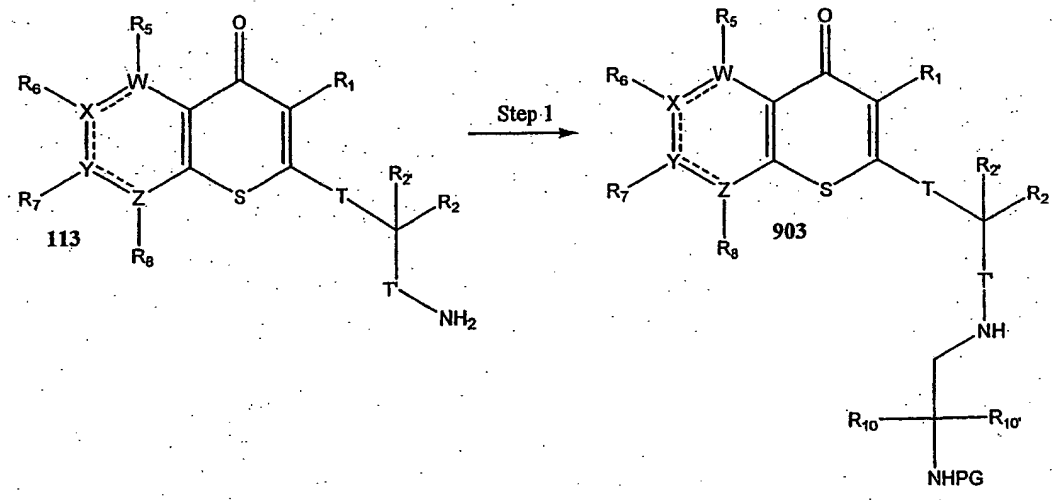
Preparation of Formula 807

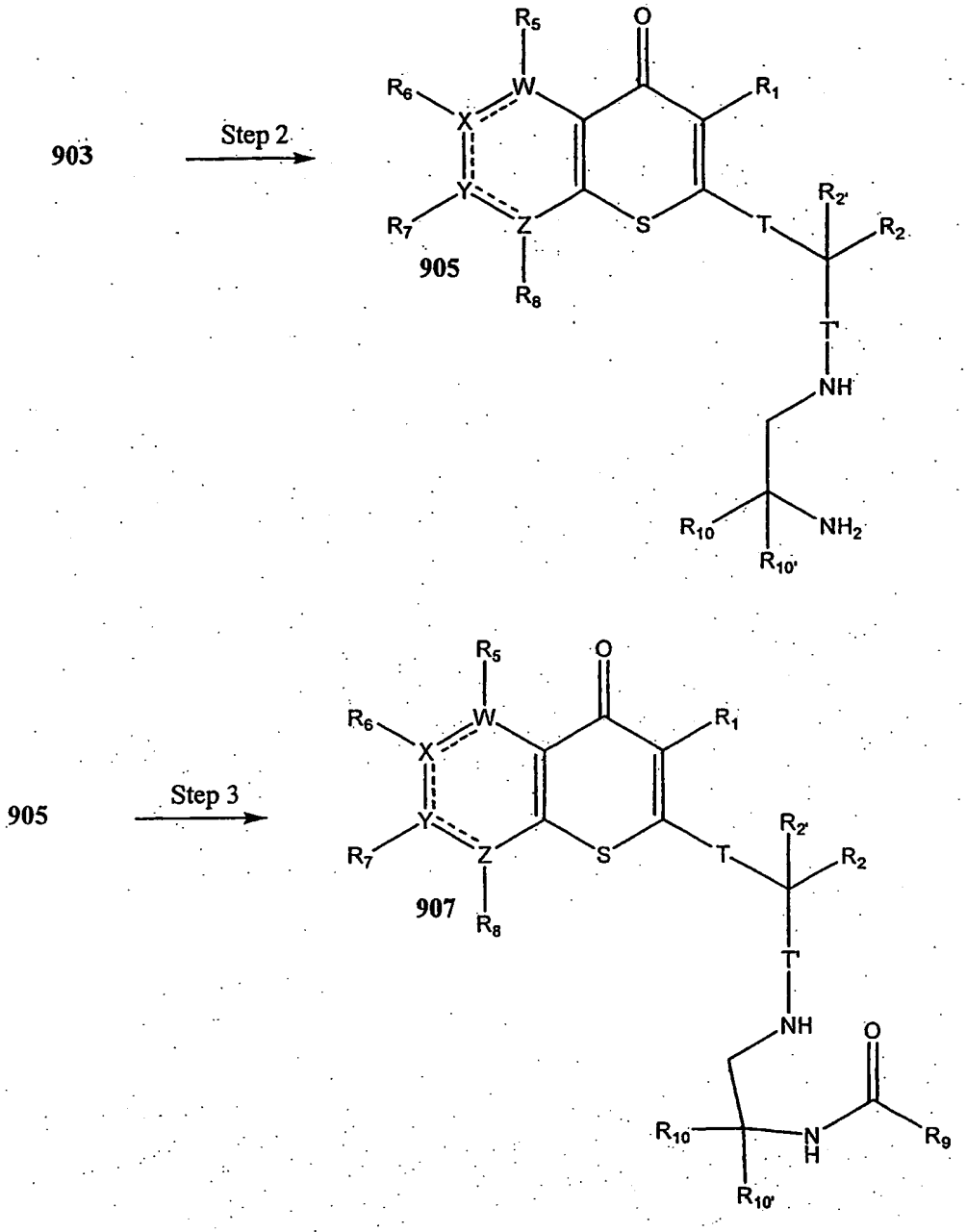
[0093] Referring to Reaction Scheme 8, Step 3, a solution of a compound of Formula 805 and an excess of ammonium acetate in acetic acid is heated at reflux using a Dean-Stark trap and condenser. Completion is monitored, e.g., by TLC. The

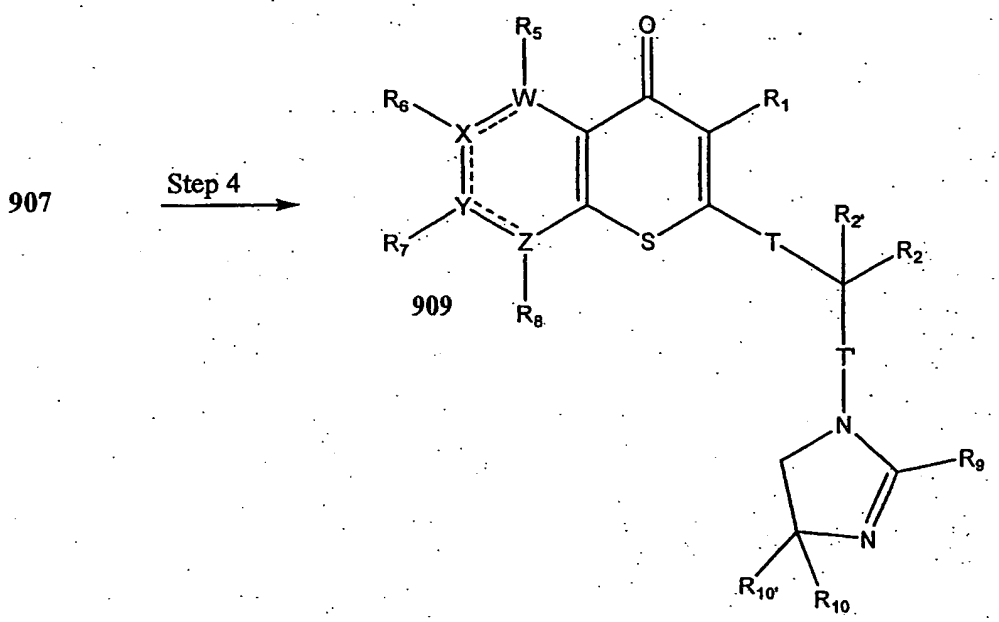
corresponding compound of Formula 807 is isolated and purified.

[0094] Optionally, when R_{10} comprises a phthalimide protecting group, the protecting group is removed as follows. A solution of a compound of Formula 907 and an excess of anhydrous hydrazine in a polar, protic solvent such as ethanol is heated at reflux. The reaction is cooled to about 5°C and any precipitate is filtered off. The filtrate is concentrated in vacuo and purified to yield the corresponding free amine. One of skill in the art will appreciate that other conditions may be used to remove other protecting groups.

Reaction Scheme 9




903
Step 2
905
Step 3
907



Preparation of Formula 903

[0095] Referring to Reaction Scheme 9, Step 1, reductive amination of amines of Formula 113 with an optionally substituted, aldehyde-containing carbamic acid ester gives urethane intermediates. Removal of the Boc protecting group furnishes an amine of Formula 905.

[0096] More specifically, to a solution of a compound of Formula 113 and an equivalent of a suitably protected aldehyde (Seki *et. al. Chem. Pharm. Bull.* 1996, *44*, 2061) in dichloromethane is added a slight excess of a reducing agent, such as sodium triacetoxyborohydride. The resultant cloudy mixture is maintained at ambient temperature. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 903 is isolated and used in the subsequent step without purification.

Preparation of Formula 905

[0097] Referring to Reaction Scheme 9, Step 2, to a solution of a compound of Formula 903 in a nonpolar, aprotic solvent such as dichloromethane is added a strong acid such as trifluoroacetic acid. The resultant solution is maintained at ambient temperature overnight and concentrated under reduced pressure. The residue is isolated to give a compound of Formula 905 which was used in the subsequent step without purification.

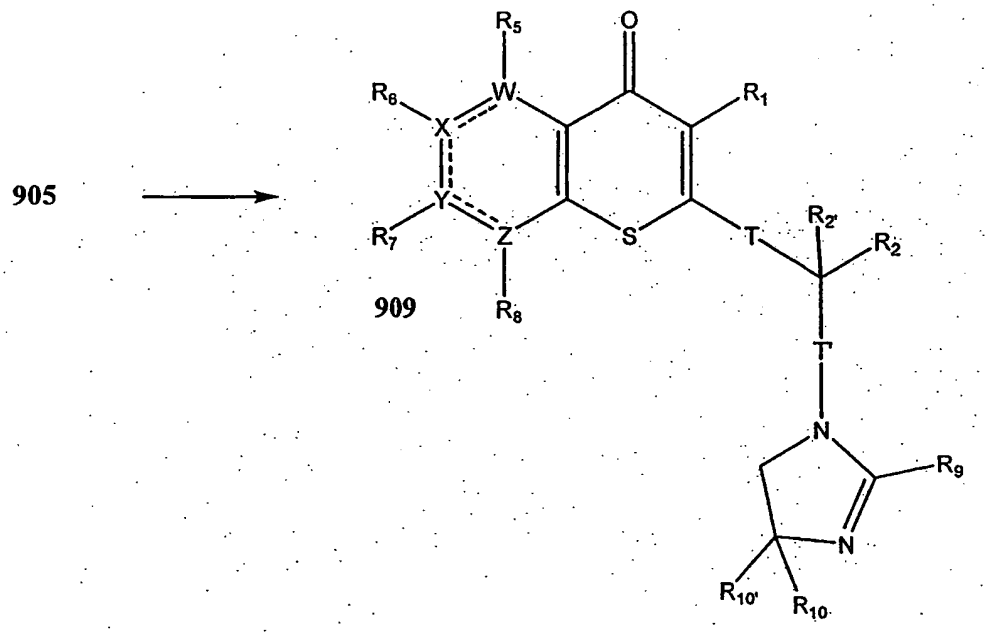
Preparation of Formula 907

[0098] Referring to Reaction Scheme 9, Step 3, to a solution of a compound of Formula 1005 in a nonpolar, aprotic solvent such as dichloromethane is added an excess, preferably about two equivalents of an amine base such as triethylamine, followed by about an equivalent or slight excess of an acid chloride. The resultant solution is stirred at ambient temperature for about 3 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 907 is isolated and purified.

Preparation of Formula 909

[0099] Referring to Reaction Scheme 9, Step 4, a solution of a compound of Formula 907 in an excess of phosphorus oxychloride is heated at reflux. After 8 hours, the reaction mixture is allowed to cool to ambient temperature and concentrated under reduced pressure. The corresponding compound of Formula 909 is isolated and purified.

Reaction Scheme 10

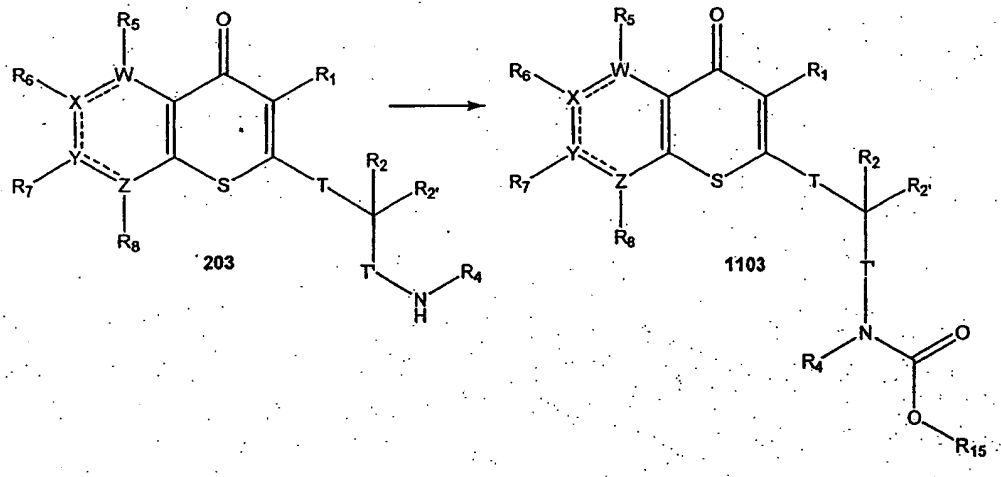


Preparation of Formula 1009

[00100] As an alternative to Steps 3 and 4 of Reaction Scheme 9, acylation of primary amines of Formula 905, followed by acetic acid mediated cyclization, can proceed without isolation of the intermediate amides to provide the target compound of Formula 909. This route is shown in Reaction Scheme 10.

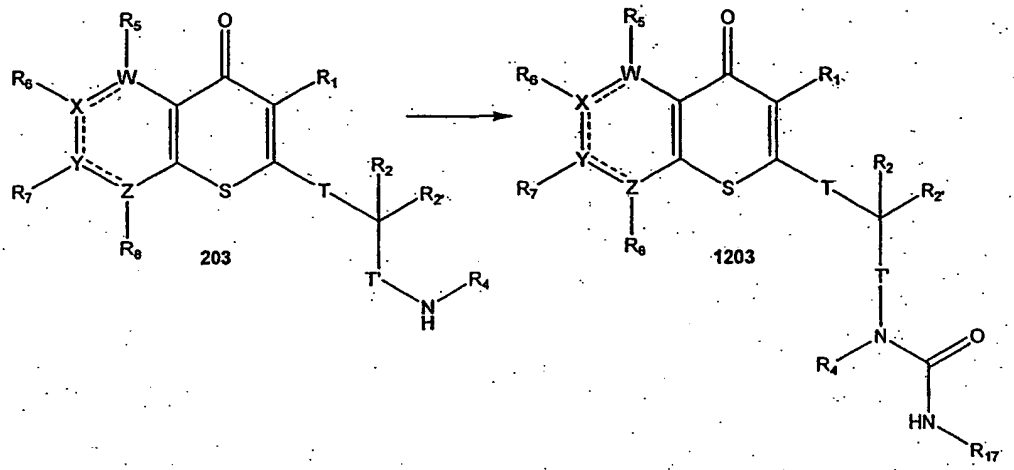
[00101] More specifically, to a solution of a compound of Formula 905 in a nonpolar, aprotic solvent such as dichloromethane is added an excess, preferably about two equivalents of an amine base, such as triethylamine, followed by about an equivalent of an acid chloride. The resultant solution is stirred at ambient temperature for 2 hours, then evaporated under reduced pressure. The resultant solid is treated with glacial acetic acid, then the resultant suspension is heated at reflux for about 48 hours. The reaction is cooled to ambient temperature then evaporated under reduced pressure. The corresponding compound of Formula 909 is isolated and purified.

Reaction Scheme 11



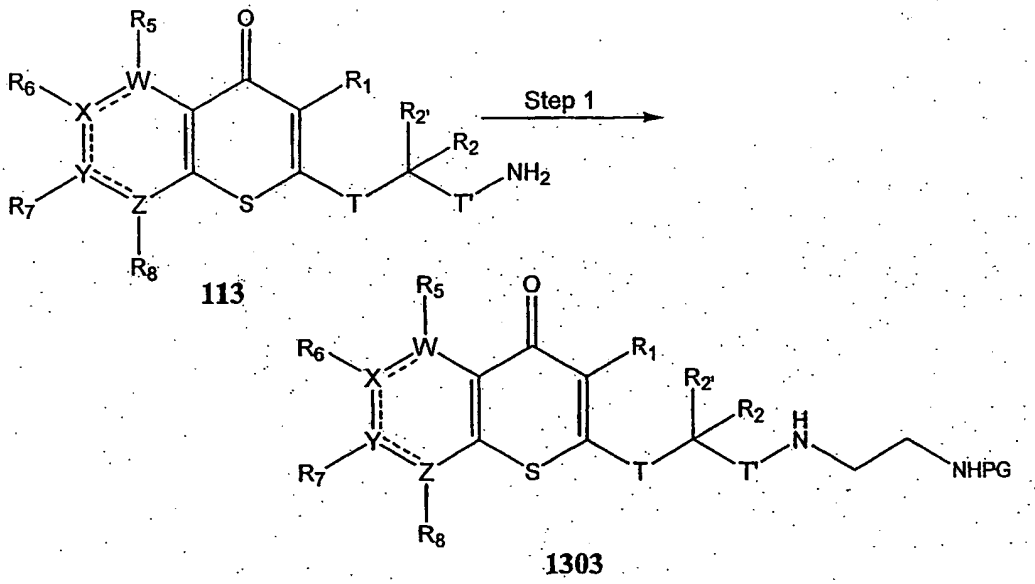
[00102] Referring to Reaction Scheme 11, a compound of Formula 203 is reacted with a slight excess of a compound of the formula $R_{15}O(CO)Cl$ in the presence of a base such as triethylamine in a nonpolar, aprotic solvent such as dichloromethane. The product, a compound of Formula 1103 is isolated and purified.

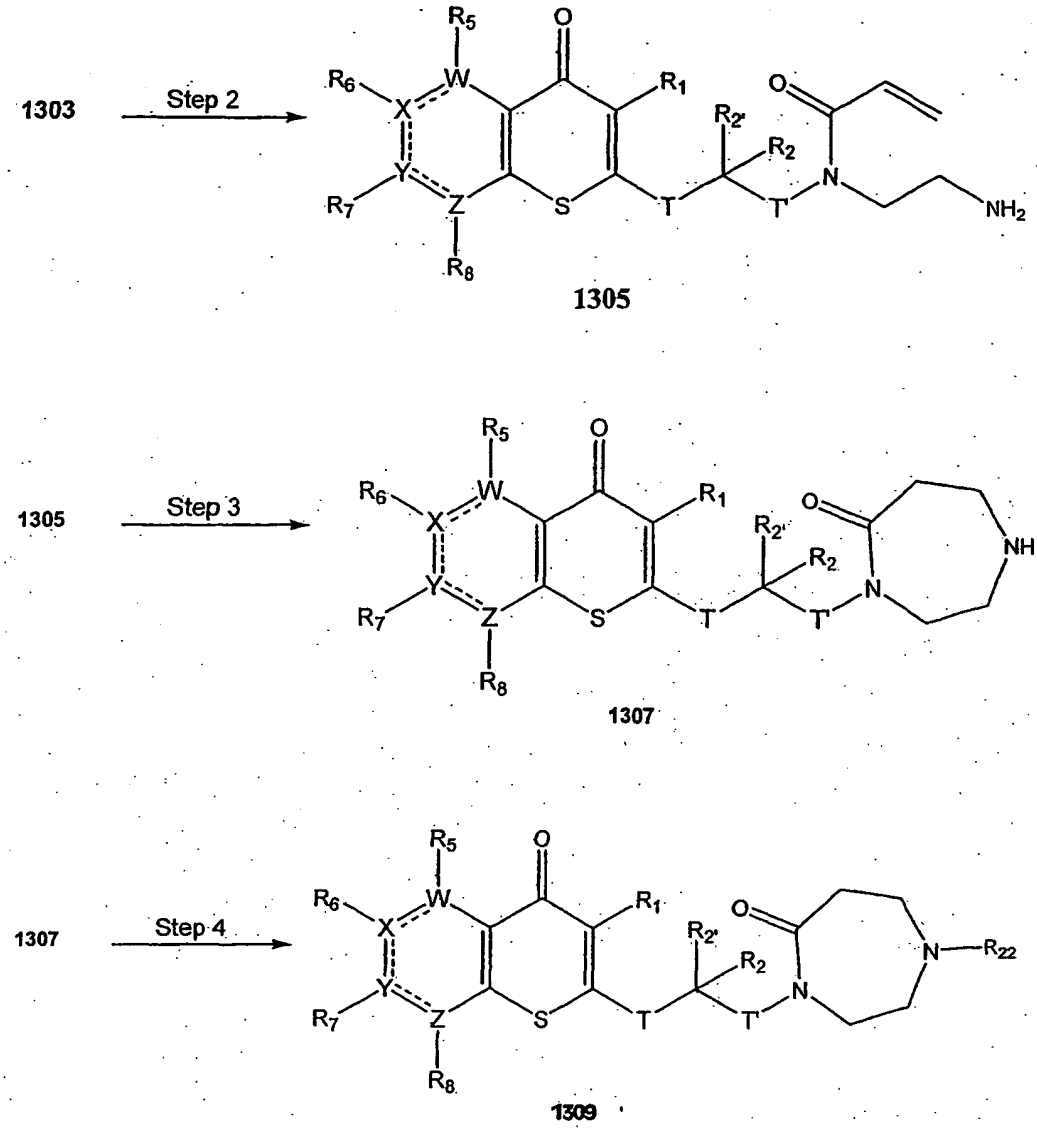
Reaction Scheme 12



[00103] Referring to Reaction Scheme 12, a compound of Formula 1203 is treated with a slight excess of an isocyanate R_{17}-N=C=O in the presence of a base, such as triethylamine, in a nonpolar, aprotic solvent, such as dichloromethane. The product, a compound of Formula 1203, is isolated and purified.

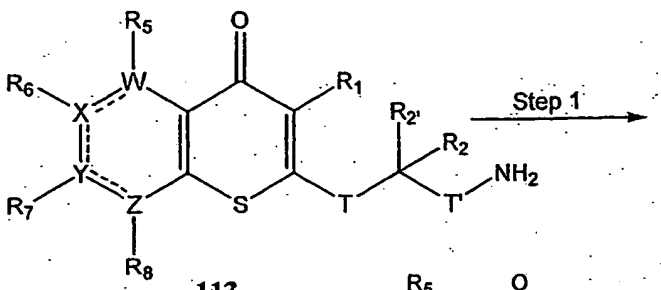
Reaction Scheme 13





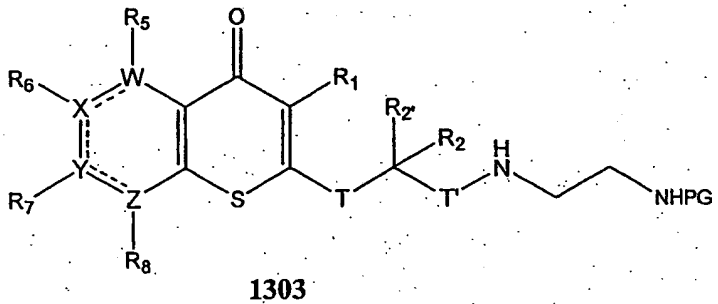
[00104] Referring to Reaction Scheme 13, reductive amination of the primary amino group in compounds of Formula 113 with (2-oxo-ethyl)-carbamic acid *tert*-butyl ester gave the corresponding secondary amine. Acylation with acryloyl chloride followed by deprotection of the tertiary amine and base mediated cyclisation gave the desired diazepanones. If desired, further functionalization of the basic amine could be accomplished under conditions well known to those skilled in the art.

Reaction Scheme 14



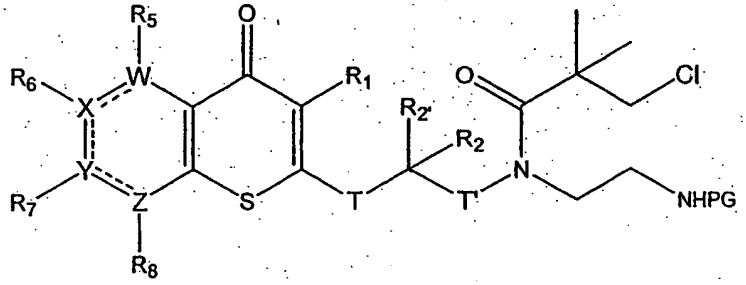
Step 1

113



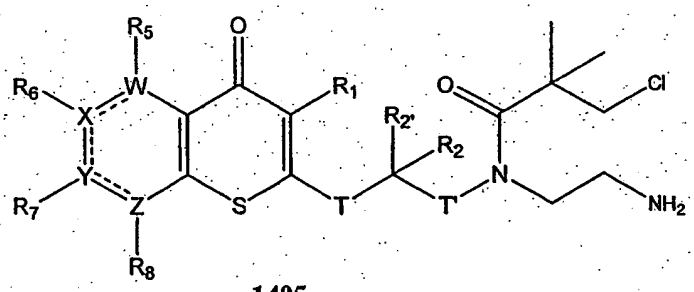
1303

1303 Step 2

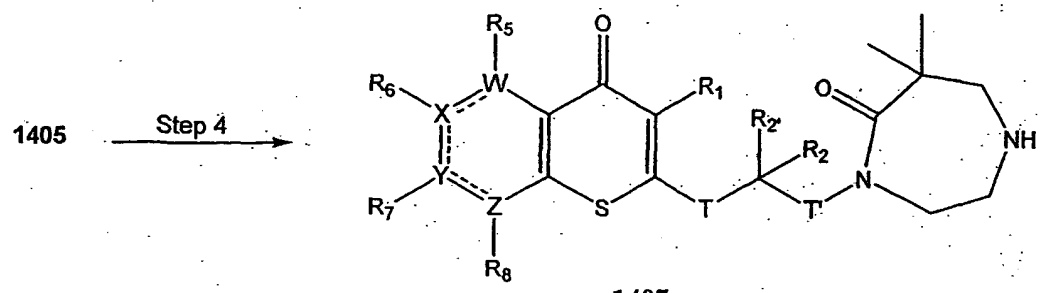


1403

1403 Step 3

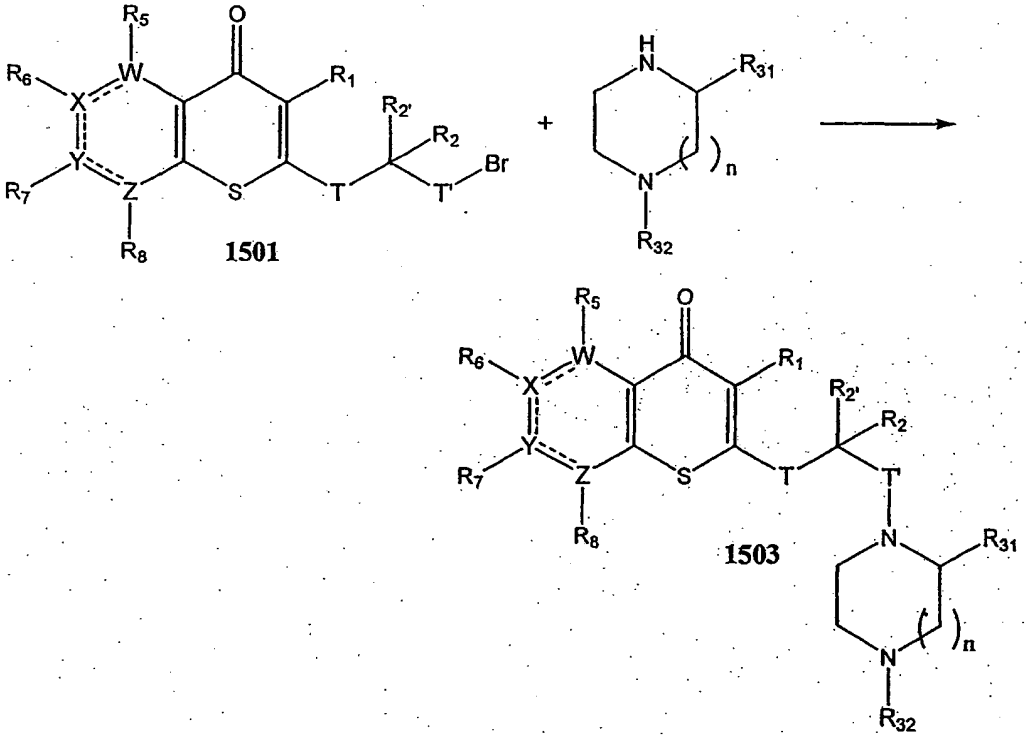


1405



[00105] Referring to Reaction Scheme 14, reductive amination of the primary amino group in compounds of Formula 113 with (2-oxo-ethyl)-carbamic acid *tert*-butyl ester gave the corresponding secondary amine. Acylation with chloropivaloyl chloride followed by deprotection of the tertiary amide and base mediated cyclisation gave the desired diazepanones. If desired, further functionalization of the basic amine could be accomplished under conditions well known to those skilled in the art.

Reaction Scheme 15



[00106] Referring to Reaction Scheme 15, a compound of Formula 1501, one-

half molar equivalent of an optionally substituted piperazine or diazepam (as shown above, where R_{32} is as described herein) and an excess of potassium carbonate are combined in an organic solvent (e.g., acetonitrile). The reaction takes place under a nitrogen atmosphere at elevated temperature (e.g., 100°C) over a period of 8 hours, followed at a somewhat lower temperature (e.g., 60°C) for a period of 5 days. The product, a compound of Formula 1503, is isolated and purified.

[00107] Optionally, in the event that R_{32} is am amine protecting group, such as Boc, it may be removed by for example treatment with a 95/5 mixture of TFA/water followed by stirring at room temperature for 1 hour. The product, a compound of Formula 1503 wherein R_{32} is hydrogen, can be isolated and purified. If desired, further functionalization of the basic amine could be accomplished under conditions well known to those skilled in the art.

Processes and Last Steps

[00108] A compound of Formula I is optionally contacted with a pharmaceutically acceptable acid or base to form the corresponding acid or base addition salt.

[00109] A pharmaceutically acceptable acid addition salt of a compound of Formula I is optionally contacted with a base to form the corresponding free base of Formula I.

[00110] A pharmaceutically acceptable base addition salt of a compound of Formula I is optionally contacted with an acid to form the corresponding free acid of Formula I.

Particular Embodiments of Compounds of the Invention

W, X, Y, and Z

[00111] When considering the compounds of Formula I, in one embodiment, W, X, Y and Z are independently chosen from –C= and –N=. In another embodiment, the ring incorporating W, X, Y, and optionally Z is an optionally substituted pyridinyl-, pyrimidinyl-, pyrazolyl-, thiazolyl-, or oxazolyl ring-. In another embodiment, W, X, Y and Z are –C=.

[00112] The dashed lines in the structure depict optional double bonds. Accordingly, in a particular embodiment, the ring comprising W, X, Y, and optionally

Z is an optionally substituted aromatic or optionally substituted heteroaromatic ring. In another embodiment, the ring is not aromatic or heteroaromatic.

T and T'

[00113] When considering the compounds of Formula I, T is optionally substituted alkylene, -C(O)-, or is a covalent bond (i.e., is absent); and T' is optionally substituted alkylene, -C(O)-, or is absent. In one embodiment, one of T and T' is absent (i.e., is a covalent bond) and the other is optionally substituted alkylene (especially optionally substituted methylene). In another embodiment, both are absent.

R_1

[00114] When considering the compounds of Formula I, in a particular embodiment, R_1 is selected from hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted phenyl-C_1-C_4-alkyl-, optionally substituted heteroaryl- C_1-C_4-alkyl-, optionally substituted naphthalenylmethyl, optionally substituted phenyl, or naphthyl (especially, optionally substituted aryl and optionally substituted aryl-C_1-C_4-alkyl-). In a more particular embodiment R_1 is selected from hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted phenyl-C_1-C_4-alkyl-, optionally substituted naphthalenylmethyl, optionally substituted phenyl, and naphthyl. More particularly, R_1 is optionally substituted phenyl-C_1-C_4-alkyl- or optionally substituted heteroaryl-C_1-C_4-alkyl-.

[00115] In a most particular embodiment R_1 is naphthyl, phenyl, bromophenyl, chlorophenyl, methoxyphenyl, ethoxyphenyl, tolyl, dimethylphenyl, chorofluorophenyl, methylchlorophenyl, ethylphenyl, phenethyl, benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, hydroxybenzyl, dichlorobenzyl, dimethoxybenzyl, or naphthalenylmethyl. More particularly, R_1 is benzyl, cyanobenzyl, methoxybenzyl, or naphthalenylmethyl. Most particularly, R_1 is benzyl.

R_2

[00116] When considering the compounds of Formula I and as will be appreciated by those skilled in the art, the compounds described herein possess a potentially chiral center at the carbon to which R_2 and $R_{2'}$ are attached. The R_2 and $R_{2'}$

groups may be the same or different; if different, the compound is chiral (i.e., has a stereogenic center). When R_2 and $R_{2'}$ are different, in particular embodiments $R_{2'}$ is hydrogen and R_2 is other than hydrogen. The invention contemplates the use of pure enantiomers and mixtures of enantiomers, including racemic mixtures, although the use of a substantially optically pure enantiomer will generally be preferred. The term "substantially optically pure" or "enantiomerically pure" means having at least about 95% of the described enantiomer with no single impurity greater than about 1% and particularly, at least about 97.5% enantiomeric excess. In a particular embodiment, the stereogenic center to which R_2 and $R_{2'}$ are attached is of the R configuration.

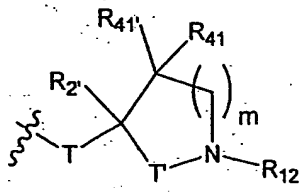
[00117] In one embodiment, R_2 is optionally substituted C_1-C_4 alkyl, and $R_{2'}$ is hydrogen or optionally substituted C_1-C_4 alkyl. More particularly, $R_{2'}$ is hydrogen and R_2 is optionally substituted C_1-C_4 alkyl. In a most particular embodiment R_2 is chosen from methyl, ethyl, propyl (particularly, c-propyl or i-propyl), butyl (particularly, t-butyl), methylthioethyl, methylthiomethyl, aminobutyl, (CBZ)aminobutyl, cyclohexylmethyl, benzyloxymethyl, methylsulfinylethyl, methylsulfinylmethyl, and hydroxymethyl, and $R_{2'}$ is hydrogen. Especially preferred is when $R_{2'}$ is hydrogen and R_2 is ethyl or propyl (particularly, c-propyl or i-propyl). More particularly, R_2 is i-propyl. More preferred is the embodiment wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration.

[00118] In another embodiment, both R_2 and $R_{2'}$ are hydrogen.

R_2 taken together with R_4

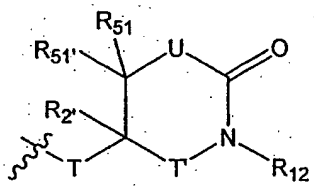
[00119] In another embodiment, R_2 and R_4 taken together form a 5- to 12-membered ring which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring and may optionally be substituted one or more of the following groups: hydroxyl, halogen (particularly chloro and fluoro), optionally substituted C_1-C_4 alkyl- (particularly methyl-), C_1-C_4 alkoxy (particularly methoxy), cyano, amino, substituted amino, oxo, or carbamyl; and $R_{2'}$ is as defined above. In a particular embodiment, R_4 is C_1-C_4 alkyl- that is optionally substituted with an oxo group.

[00120] In a particular embodiment, R_2 and R_4 taken together form an optionally substituted ring of the formula:



wherein R_{41} and $R_{41'}$ are independently chosen from hydrogen, alkyl, aryl, aralkyl, heteroaryl, substituted alkyl, substituted aryl, substituted aralkyl, and substituted heteroaryl; m is 0, 1, 2, or 3; and T, T', R_{12}, and $R_{2'}$ are as defined above. In a more particular embodiment, R_{41} is hydrogen. In another particular embodiment, both R_{41} and $R_{41'}$ are hydrogen. In another embodiment, R_{12} is optionally substituted aralkyl (especially benzyl) or optionally substituted acyl (especially p-methyl-benzoyl). See, e.g., USSN 60/414,756, which is incorporated herein by reference for all purposes.

[00121] In another embodiment, R_2 and R_4 taken together form an optionally substituted ring of the formula:



wherein R_{12}, $R_{2'}$, T, and T' are as defined above; R_{51} and $R_{51'}$ are independently chosen from hydrogen, alkyl, aryl, aralkyl, heteroaryl, substituted alkyl, substituted aryl, substituted aralkyl and substituted heteroaryl; U is a covalent bond, CR'R" or NR'"; R' and R" are independently chosen from hydrogen, hydroxy, amino, optionally substituted aryl, optionally substituted alkylamino, optionally substituted alkyl and optionally substituted alkoxy; and R'" is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl, provided that U is not a covalent bond when T' is absent.

[00122] In a particular embodiment, R_{51} is hydrogen or optionally substituted lower alkyl; more particularly, R_{51} is hydrogen. In another embodiment, $R_{51'}$ is hydrogen or optionally substituted lower alkyl; more particularly, $R_{51'}$ is hydrogen.

[00123] In one embodiment, R_{12} is optionally substituted aryl or optionally

substituted aralkyl; more particularly, R_{12} is optionally substituted phenyl, benzyl or methyl-benzyl (especially, benzyl or methyl-benzyl). In another embodiment, R_{12} is $-C(O)R_3$ and R_3 is optionally substituted aryl or optionally substituted aralkyl.

[00124] In one embodiment, U is CR'R" where R' and/or R" are hydrogen. In another embodiment, U is NR'" where R'" is hydrogen or optionally substituted alkyl. More particularly, R'" is hydrogen or optionally substituted amino-lower alkyl. See, e.g., USSN 60/398,224, which is incorporated herein by reference for all purposes.

R_{12}

[00125] In one embodiment, R_{12} is chosen from C_1-C_{13} alkyl; substituted lower alkyl; phenyl; naphthyl; phenyl substituted with halo, lower alkyl, lower alkoxy, nitro, methylenedioxy, or trifluoromethyl; biphenylyl, benzyl and heterocyclyl. More particularly, R_{12} is chosen from substituted phenyl, heterocyclyl and naphthyl. Most particularly, R_{12} is chosen from halophenyl, polyhalophenyl, tolyl, dimethylphenyl, methoxyphenyl, dimethoxyphenyl, cyanophenyl, trifluoromethylphenyl, trifluoromethoxyphenyl, bis(trifluoromethyl)phenyl, carboxyphenyl, t-butylphenyl, methoxycarbonylphenyl, piperidinyl and naphthyl.

R_3 Groups When R_{12} is $-C(O)R_3$

[00126] When considering the compounds of Formula I wherein R_{12} is $-C(O)R_3$, in a particular embodiment R_3 is selected from optionally substituted C_1-C_8 alkyl, optionally substituted aryl-C_1-C_4-alkyl-, optionally substituted heteroaryl-C_1-C_4-alkyl-, optionally substituted heteroaryl, optionally substituted aryl, $R_{15}O$- and R_{17}-NH-, R_{15} is chosen from optionally substituted C_1-C_8 alkyl and optionally substituted aryl, and R_{17} is chosen from hydrogen, optionally substituted C_1-C_8 alkyl and optionally substituted aryl. Particular R_3 are optionally substituted C_1-C_8 alkyl (e.g., C_1-C_8 alkyl substituted with lower-alkoxy), optionally substituted heteroaryl, and optionally substituted aryl.

[00127] In a more particular embodiment, when R_3 is not $R_{17}NH$- or $R_{15}O$-, R_3 is chosen from phenyl; phenyl substituted with one or more of the following substituents: halo, C_1-C_4 alkyl, C_1-C_4 alkyl substituted with hydroxy (e.g., hydroxymethyl), C_1-C_4 alkoxy, C_1-C_4 alkyl substituted with C_1-C_4 alkoxy, nitro, formyl, carboxy, cyano, methylenedioxy, ethylenedioxy, acyl (e.g., acetyl), -N-acyl

(e.g., N-acetyl) or trifluoromethyl; benzyl; phenoxymethyl-; halophenoxymethyl-; phenylvinyl-; heteroaryl-; heteroaryl- substituted with C_1-C_4 alkyl or C_1-C_4 alkyl substituted with halo (e.g., CF_3); C_1-C_4 alkyl substituted with C_1-C_4 alkoxy- and benzyloxymethyl-.

[00128] In a most particular embodiment, when R_3 is not $R_{17}NH$- or $R_{15}O$-, R_3 is chosen from phenyl, halophenyl, dihalophenyl, cyanophenyl, halo(trifluoromethyl)phenyl, hydroxymethylphenyl, methoxyphenyl, ethoxyphenyl, carboxyphenyl, ethylphenyl, tolyl, methylenedioxyphenyl, ethylenedioxyphenyl, methoxychlorophenyl, dihydro-benzodioxinyl, methylhalophenyl, trifluoromethylphenyl, bis(trifluoromethyl)phenylbenzyl, furanyl, C_1-C_4 alkyl substituted furanyl, trifluoromethylfuranyl, C_1-C_4 alkyl substituted trifluoromethylfuranyl, benzofuranyl, thiophenyl, C_1-C_4 alkyl substituted thiophenyl, benzothiophenyl, benzothiadiazolyl, pyridinyl, indolyl, methylpyridinyl, trifluoromethylpyridinyl, pyrrolyl, quinolinyl, picolinyl, pyrazolyl, C_1-C_4 alkyl substituted pyrazolyl, N-methyl pyrazolyl, C_1-C_4 alkyl substituted N-methyl pyrazolyl, C_1-C_4 alkyl substituted pyrazinyl, C_1-C_4 alkyl substituted isoxazolyl, benzoisoxazolyl, morpholinomethyl, methylthiomethyl, methoxymethyl, N-methyl imidazolyl, and imidazolyl. Yet more particularly, R_3 is tolyl, halophenyl, halomethylphenyl, hydroxymethylphenyl, methylenedioxyphenyl, formylphenyl or cyanophenyl.

[00129] In a more particular embodiment, when R_3 is $R_{17}NH$-, R_{17} is chosen from hydrogen, C_1-C_4 alkyl; cyclohexyl; phenyl; and phenyl substituted with halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, or C_1-C_4 alkylthio.

[00130] In a most particular embodiment, when R_3 is $R_{17}NH$-, R_{17} is hydrogen isopropyl, butyl, cyclohexyl, phenyl, bromophenyl, dichlorophenyl, methoxyphenyl, ethylphenyl, tolyl, trifluoromethylphenyl or methylthiophenyl.

[00131] In an embodiment, wherein R_3 is $R_{15}O$-, R_{15} is chosen from optionally substituted C_1-C_8 alkyl and optionally substituted aryl.

R_{3a} Groups when R_{12} is $-SO_2R_{3a}$

[00132] In one embodiment, when R_{12} is $-SO_2R_{3a}$, R_{3a} is chosen from C_1-C_{13} alkyl; phenyl; naphthyl; phenyl substituted with halo, C_1-C_4 alkyl, C_1-C_4 alkoxy,

cyano, nitro, methylenedioxy, or trifluoromethyl; biphenylyl and heteroaryl. More particularly, R_{3a} is chosen from phenyl substituted with halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, cyano, nitro, methylenedioxy, or trifluoromethyl and naphthyl.

R_4 Groups

[00133] In a particular embodiment, R_4 is chosen from hydrogen, optionally substituted C_1-C_{13} alkyl, optionally substituted aryl, optionally substituted aryl-C_1-C_4-alkyl-, optionally substituted heterocyclyl, and optionally substituted heteroaryl-C_1-C_4-alkyl- (especially hydrogen or optionally substituted C_1-C_{13} alkyl).

[00134] More particularly, R_4 is chosen from hydrogen, C_1-C_4 alkyl; cyclohexyl; phenyl substituted with hydroxyl, C_1-C_4 alkoxy or C_1-C_4 alkyl; benzyl; heteroarylmethyl-; heteroarylethyl-; heteroarylpropyl-; and R_{16}-alkylene-, wherein R_{16} is hydroxyl, di(C_1-C_4 alkyl)amino-, (C_1-C_4 alkyl)amino-, amino, C_1-C_4 alkoxy-, or N-heterocyclyl-, particularly pyrrolidino, piperidino or imidazolyl.

[00135] More particularly, R_4 is R_{16}-alkylene-, wherein R_{16} is amino, C_1-C_4 alkylamino-, di(C_1-C_4 alkyl)amino-, C_1-C_4 alkoxy-, hydroxyl, or N-heterocyclyl. Particularly R_{16} is amino.

[00136] In a most particular embodiment, R_4 is chosen from hydrogen, methyl, ethyl, propyl, butyl, cyclohexyl, carboxyethyl, carboxymethyl, methoxyethyl, hydroxyethyl, hydroxypropyl, dimethylaminoethyl, dimethylaminopropyl, diethylaminoethyl, diethylaminopropyl, aminopropyl, methylaminopropyl, 2,2-dimethyl-3-(dimethylamino)propyl, 1-cyclohexyl-4-(diethylamino)butyl, aminoethyl, aminobutyl, aminopentyl, aminohexyl, aminoethoxyethyl, isopropylaminopropyl, diisopropylaminoethyl, 1-methyl-4-(diethylamino)butyl, (t-Boc)aminopropyl, hydroxyphenyl, benzyl, methoxyphenyl, methylmethoxyphenyl, dimethylphenyl, tolyl, ethylphenyl, (oxopyrrolidinyl)propyl, (methoxycarbonyl)ethyl, benzylpiperidinyl, pyridinylethyl, pyridinylmethyl, morpholinylethyl morpholinylpropyl, piperidinyl, azetidinylmethyl, azetidinylethyl, azetidinylpropyl pyrrolidinylethyl, pyrrolidinylpropyl, piperidinylmethyl, piperidinylethyl, imidazolylpropyl, imidazolylethyl, (ethylpyrrolidinyl)methyl, (methylpyrrolidinyl)ethyl, (methylpiperidinyl)propyl, (methylpiperazinyl)propyl, furanylmethyl and indolylethyl.

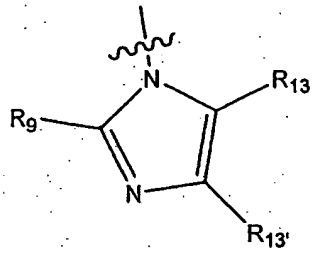
[00137] More particularly, R_4 is aminoethyl, aminopropyl, aminobutyl,

aminopentyl, aminohexyl, methylaminoethyl, methylaminopropyl, methylaminobutyl, methylaminopentyl, methylaminohexyl, dimethylaminoethyl, dimethylaminopropyl, dimethylaminobutyyl, dimethylaminopentyl, dimethylaminohexyl, ethylaminoethyl, ethylaminopropyl, ethylaminobutyl, ethylaminopentyl, ethylaminohexyl, diethylaminoethyl, diethylaminopropyl, diethylaminobutyyl, diethylaminopentyl, or diethylaminohexyl, most particularly aminopropyl.

R_{12} taken together with R_4

[00138] When considering the compounds of Formula I, in one embodiment, R_4 taken together with R_{12}, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring.

[00139] In a particular embodiment, R_4 taken together with R_{12} and the nitrogen to which they are bound, forms an optionally substituted imidazolinyl ring of the formula:

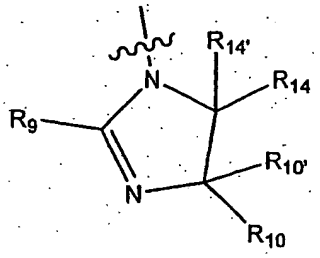


wherein

R_9 is chosen from hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, optionally substituted aryl-C_1-C_4-alkyl -, optionally substituted heteroaryl-C_1-C_4-alkyl -, optionally substituted aryl-C_1-C_4-alkoxy -, optionally substituted heteroaryl-C_1-C_4-alkoxy -, optionally substituted heteroaryl-; and R_{13} and $R_{13'}$ are independently hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, or optionally substituted aryl-C_1-C_4-alkyl - (especially optionally substituted aryl). More particularly, R_9 is phenyl substituted with C_1-C_4-alkyl, C_1-C_4-alkoxy-, and/or halo (especially C_1-C_4-alkyl and/or halo); phenyl; or benzyl. Yet more particularly, R_9 is tolyl; halophenyl; or halomethylphenyl.

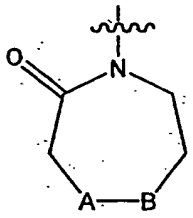
[00140] In a particular embodiment, R_{13} is hydrogen and $R_{13'}$ is substituted C_1-C_4 alkyl. More particularly, R_{13} is hydrogen and $R_{13'}$ is aminomethyl, aminoethyl, aminopropyl, acetylamino-methyl, acetylaminoethyl, benzyloxycarbonylamino-methyl or benzyloxycarbonylamino-ethyl. See, e.g., PCT/US03/14787, which is incorporated herein by reference.

[00141] In another particular embodiment, R_{12} taken together with R_4 forms an optionally substituted imidazolinyl ring of the formula:



wherein R_9 is chosen from hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, optionally substituted aryl-C_1-C_4-alkyl -, and optionally substituted heteroaryl-; and R_{10}, $R_{10'}$, R_{14}, and $R_{14'}$ are independently chosen from hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, and optionally substituted aryl-C_1-C_4-alkyl -. More particularly, R_9 is methylenedioxyphenyl; phenyl; phenyl substituted with C_1-C_4 alkyl, C_1-C_4 alkoxy, and/or halo; or benzyl. In a particular embodiment, R_9 is methylenedioxyphenyl-; phenyl; or phenyl substituted with methoxy, halo and/or methyl (especially halo and/or methyl, including tolyl), more particularly methylenedioxyphenyl or said substituted phenyls. In another particular embodiment, R_{10}, $R_{10'}$, $R_{14'}$, and R_{14} are independently hydrogen or optionally substituted alkyl (especially optionally substituted C_1-C_4 alkyl). More particularly, R_{10} and $R_{10'}$ are independently selected from the group consisting of hydrogen or optionally substituted C_1-C_4 alkyl (and more particularly, methyl or aminoalkyl-) and $R_{14'}$ and R_{14} are hydrogen.

[00142] In another embodiment, R_4 taken together with R_{12} forms an optionally substituted diazepinone ring of the formula:

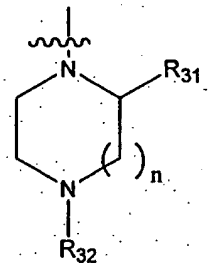


wherein A and B are each independently chosen from $C(R_{20})(R_{21})$, $N(R_{22})$, O or S, wherein R_{20} and R_{21} are each independently selected from H, optionally substituted alkyl optionally substituted aryl and optionally substituted heteroaryl; and R_{22} is H, optionally substituted alkyl, optionally substituted aralkyl, optionally substituted heteroaralkyl, optionally substituted alkylcarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroarylcarbonyl, optionally substituted aralkylcarbonyl, optionally substituted heteroaralkylcarbonyl, optionally substituted alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted heteroaryloxycarbonyl, optionally substituted aralkyloxycarbonyl, optionally substituted heteroaralkyloxycarbonyl. In a more particular embodiment, the diazepinone ring is further substituted with one or more of the following groups: optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl.

[00143] In yet another embodiment of the compounds of Formula I, one of A or B is $C(R_{20})(R_{21})$, wherein R_{20} and R_{21} are each independently selected from H or C_1-C_4 alkyl, and the other of A or B is $N(R_{22})$, where R_{22} is H, C_1-C_4 alkyl, optionally substituted aralkyl, optionally substituted heteroaralkyl, C_1-C_6 alkylcarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroarylcarbonyl, optionally substituted aralkylcarbonyl, optionally substituted heteroaralkylcarbonyl, C_1-C_6 alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted heteroaryloxycarbonyl, optionally substituted aralkyloxycarbonyl, optionally substituted heteroaralkyloxycarbonyl, where the optionally substituted aryl or heteroaryl groups or moieties are unsubstituted or substituted with one or more substituents selected from C_1-C_4 alkyl, C_1-C_4 haloalkyl, C_1-C_4 alkoxy, C_1-C_4 haloalkoxy, amino, C_1-C_4 alkylamino, di-C_1-C_4 alkylamino, carboxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkoxycarbonyl, carboxamido, C_1-C_4 alkylcarboxamido, aminocarbonyl, C_1-C_4 alkylaminocarbonyl,

di-C_1-C_4 alkylaminocarbonyl, cyano, C_1-C_4 alkylcarbonyl, halogen, hydroxyl, mercapto and nitro. In another embodiment, A is $C(R_{20})(R_{21})$, wherein R_{20} and R_{21} are each H or C_1-C_4 alkyl, and B is $N(R_{22})$, where R_{22} is H, C_1-C_4 alkyl, aralkyl, heteroaralkyl, C_1-C_6 alkylcarbonyl, arylcarbonyl, heteroarylcarbonyl. In specific embodiments of the compounds of Formula I, A is CH_2, and B is $N(R_{22})$, where R_{22} is H, methyl, benzyl or acetyl (-C(O)methyl). See, e.g., USSN 60/435,001, which is incorporated herein by reference for all purposes.

[00144] In another embodiment, R_4 taken together with R_{12} forms an optionally substituted piperazine- or diazepam of the formula:



R_{31} and R_{32} are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aralkyl, and optionally substituted heteroaralkyl; and n is 1 or 2. More particularly, R_{31} is aryl (especially phenyl), substituted aryl (especially lower alkyl-, lower alkoxy-, and/or halo-substituted phenyl), aralkyl (especially benzyl and phenylvinyl), heteroaralkyl, substituted aralkyl (especially substituted benzyl and substituted phenylvinyl), or substituted heteroaralkyl; R_{32} is hydrogen; and n is 1. See, e.g., USSN 60/404,864, which is incorporated herein by reference.

R_5, R_6, R_7, and R_8 Groups

[00145] When considering the compounds of Formula I, R_5, R_6, R_7 and R_8 are independently chosen from hydrogen; acyl; alkyl; alkyl substituted with alkyl, alkoxy, halo, hydroxyl, nitro, cyano, alkylamino, dialkylamino, alkylsulfonyl, alkylsulfonamido, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, lower-alkylaminocarbonyl- (e.g. methylaminocarbonyl- or ethylaminocarbonyl-), di(lower-alkyl)aminocarbonyl- (e.g. dimethylaminocarbonyl- or diethylaminocarbonyl-), aryl,

or heteroaryl; alkoxy; alkoxy substituted with alkyl, acyl, alkoxy, halo, hydroxyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, lower-alkylaminocarbonyl- (e.g. methylaminocarbonyl- or ethylaminocarbonyl-), di(lower-alkyl)aminocarbonyl- (e.g. dimethylaminocarbonyl- or diethylaminocarbonyl-), aryl, or heteroaryl; halogen; hydroxyl; nitro; cyano; amino, alkylamino,dialkylamino; alkylsulfonyl; alkylsulfonamido; alkylthio; aminocarbonyl; aryl; aryl substituted with alkyl, acyl, alkoxy, halo, hydroxyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, lower-alkylaminocarbonyl- (e.g. methylaminocarbonyl- or ethylaminocarbonyl-), di(lower-alkyl)aminocarbonyl- (e.g. dimethylaminocarbonyl- or diethylaminocarbonyl-), aryl, or heteroaryl; heteroaryl; or heteroaryl substituted with alkyl, acyl, alkoxy, halo, hydroxyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, lower-alkylaminocarbonyl- (e.g. methylaminocarbonyl- or ethylaminocarbonyl-), di(lower-alkyl)aminocarbonyl- (e.g. dimethylaminocarbonyl- or diethylaminocarbonyl-), aryl, or heteroaryl.

[00146] More particularly, R_5, R_6, R_7, and R_8 are independently chosen from hydrogen, amino, alkylamino, hydroxyl, halogen (particularly chloro and fluoro), C_1-C_4 alkyl (particularly methyl), C_1-C_4 haloalkyl (particularly trifluoromethyl), C_1-C_4 alkoxy (particularly methoxy), C_1-C_4 haloalkoxy and cyano. More particularly, R_5, R_6, R_7, and R_8 are methoxy, hydrogen, cyano, or halo (especially Cl, F). Further for each of the specific substituents: R_5 is amino, alkylamino, trifluoromethyl, hydrogen or halo; R_6 is hydrogen, alkyl (particulary, methyl) or halo; R_7 is hydrogen, halo, alkyl (particulary, methyl), alkoxy (particularly, methoxy), cyano, or trifluoromethyl; and R_8 is hydrogen or halo. Still further are the compounds where only one of R_5, R_6, R_7, and R_8 is not hydrogen, especially R_7. More particularly are the compounds where R_5, R_6, and R_8 are hydrogen and R_7 is cyano, methoxy or halogen (especially Cl, F).

Salt Forms

[00147] Compounds of the invention will generally be capable of forming acid addition salts (i.e., will comprise a site which reacts with a pharmaceutically acceptable acid to form an acid addition salt.) The present invention includes

pharmaceutically acceptable acid addition salts of the compounds of Formula I. Acid addition salts of the present compounds are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, maleic, succinic or methanesulfonic.

[00148] The salts and/or solvates of the compounds of the Formula I which are not pharmaceutically acceptable may be useful as intermediates in the preparation of pharmaceutically acceptable salts and/or solvates of compounds of formula I or the compounds of the formula I themselves, and as such form another aspect of the present invention.

Particular Subgenus

[00149] In a particular subgenus of compounds of Formula I,

W, X, Y, and Z are C;

T and T' are absent;

R_1 is optionally substituted aryl-C_1-C_4-alkyl-, optionally substituted heteroaryl-C_1-C_4-alkyl-, or naphthalenylmethyl (especially benzyl, halobenzyl, methoxylbenzyl, cyanobenzyl, or naphthylmethyl);

R_2 is optionally substituted C_1-C_4-alkyl- (especially ethyl or propyl);

$R_{2'}$ is hydrogen;

R_{12} is $-C(O)R_3$;

R_3 is selected from hydrogen, optionally substituted alkyl-, optionally substituted aralkyl-, optionally substituted heteroaralkyl-, optionally substituted heteroaryl-, optionally substituted aryl-, $R_{15}O$- and R_{17}-NH-, wherein R_{15} is chosen from optionally substituted alkyl and optionally substituted aryl and R_{17} is chosen from hydrogen, optionally substituted alkyl and optionally substituted aryl;

R_4 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaralkyl-, and optionally substituted heterocyclyl-

R_5, R_6, and R_8 are hydrogen; and

R_7 is halo, cyano, methoxy, or hydroxyl.

More particularly, R_4 is R_{16}-alkylene- wherein R_{16} is hydroxy, di(lower alkylamino), (lower alkyl)amino, amino, pyrrolidino, piperidino, imidazolyl and morpholino.

[00150] In another embodiment, W, X, Y, Z, T, T', R_1, R_2, $R_{2'}$, R_4, R_5, R_6, R_7, and R_8 are as defined above; and R_{12} is hydrogen.

[00151] In another embodiment, W, X, Y, Z, T, T', R_1, R_2, $R_{2'}$, R_5, R_6, R_7, and R_8 are as defined above; and R_{12} taken together with R_4 forms an optionally substituted imidazolinyl ring.

[00152] In another embodiment, W, X, Y, Z, T, T', R_1, R_2, $R_{2'}$, R_5, R_6, R_7, and R_8 are as defined above; and R_{12} taken together with R_4 forms an optionally substituted imidazolyl ring.

[00153] In another embodiment, W, X, Y, Z, T, T', R_1, R_2, $R_{2'}$, R_4, R_5, R_6, R_7, and R_8 are as defined above; and R_{12} is $-SO_2R_{3a}$ where R_{3a} is chosen from substituted phenyl and naphthyl.

[00154] A particular compound of the invention is N-(3-amino-propyl)-N-[1-(3-benzyl-7-chloro-4-oxo-4H-thiochromen-2-yl)-2-methyl-propyl]-4-methyl-benzamide.

Utility, Testing and Administration

General Utility

[00155] Once made, the compounds of the invention find use in a variety of applications involving alteration of mitosis. As will be appreciated by those skilled in the art, mitosis may be altered in a variety of ways; that is, one can affect mitosis either by increasing or decreasing the activity of a component in the mitotic pathway. Stated differently, mitosis may be affected (e.g., disrupted) by disturbing equilibrium, either by inhibiting or activating certain components. Similar approaches may be used to alter meiosis.

[00156] In a particular embodiment, the compounds of the invention are used to inhibit mitotic spindle formation, thus causing prolonged cell cycle arrest in mitosis. By "inhibit" in this context is meant decreasing or interfering with mitotic spindle formation or causing mitotic spindle dysfunction. By "mitotic spindle formation" herein is meant organization of microtubules into bipolar structures by mitotic kinesins. By "mitotic spindle dysfunction" herein is meant mitotic arrest and monopolar spindle formation.

[00157] The compounds of the invention are useful to bind to, and/or inhibit the activity of, a mitotic kinesin, KSP. In one embodiment, the KSP is human KSP,

although the compounds may be used to bind to or inhibit the activity of KSP kinesins from other organisms. In this context, "inhibit" means either increasing or decreasing spindle pole separation, causing malformation, i.e., splaying, of mitotic spindle poles, or otherwise causing morphological perturbation of the mitotic spindle. Also included within the definition of KSP for these purposes are variants and/or fragments of KSP. See U.S. Patent 6,437,115, hereby incorporated by reference in its entirety. The compounds of the invention have been shown to have specificity for KSP. However, the present invention includes the use of the compounds to bind to or modulate other mitotic kinesins.

[00158] The compounds of the invention are used to treat cellular proliferation diseases. Such disease states which can be treated by the compounds, compositions and methods provided herein include, but are not limited to, cancer (further discussed below), autoimmune disease, fungal disorders, arthritis, graft rejection, inflammatory bowel disease, cellular proliferation induced after medical procedures, including, but not limited to, surgery, angioplasty, and the like. Treatment includes inhibiting cellular proliferation. It is appreciated that in some cases the cells may not be in an abnormal state and still require treatment. Thus, in one embodiment, the invention herein includes application to cells or individuals afflicted or subject to impending affliction with any one of these disorders or states.

[00159] The compounds, pharmaceutical formulations and methods provided herein are particularly deemed useful for the treatment of cancer including solid tumors such as skin, breast, brain, cervical carcinomas, testicular carcinomas, etc. More particularly, cancers that can be treated include, but are not limited to:

- Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma;
- Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma;
- Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid

tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma);

- Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma);
- Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma;
- Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors;
- Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma);
- Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma], fallopian tubes (carcinoma);
- Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple

myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma];

- Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and
- Adrenal glands: neuroblastoma.

As used herein, treatment of cancer includes treatment of cancerous cells, including cells afflicted by any one of the above-identified conditions. Thus, the term "cancerous cell" as provided herein, includes a cell afflicted by any one of the above identified conditions.

[00160] Another useful aspect of the invention is a kit having a compound, salt or solvate of Formula I and a package insert or other labeling including directions treating a cellular proliferative disease by administering an effective amount of the compound, salt or solvate. The compound, salt or solvate of Formula I in the kits of the invention is particularly provided as one or more doses for a course of treatment for a cellular proliferative disease, each dose being a pharmaceutical formulation including a pharmaceutically accepted excipient and a compound, salt or solvate of Formula I.

Testing

[00161] For assay of KSP-modulating activity, generally either KSP or a compound according to the invention is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g., a microtiter plate, an array, etc.). The insoluble support may be made of any composition to which the sample can be bound, is readily separated from soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any convenient shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose, Teflon™, etc. Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the sample is not crucial so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the sample and is nondiffusable. Particular methods of binding include the use of antibodies

(which do not sterically block either the ligand binding site or activation sequence when the protein is bound to the support), direct binding to "sticky" or ionic supports, chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following binding of the sample, excess unbound material is removed by washing. The sample receiving areas may then be blocked through incubation with bovine serum albumin (BSA), casein or other innocuous protein or other moiety.

[00162] The compounds of the invention may be used on their own to inhibit the activity of a mitotic kinesin, particularly KSP. In one embodiment, a compound of the invention is combined with KSP and the activity of KSP is assayed. Kinesin (including KSP) activity is known in the art and includes one or more kinesin activities. Kinesin activities include the ability to affect ATP hydrolysis; microtubule binding; gliding and polymerization/depolymerization (effects on microtubule dynamics); binding to other proteins of the spindle; binding to proteins involved in cell-cycle control; serving as a substrate to other enzymes, such as kinases or proteases; and specific kinesin cellular activities such as spindle pole separation.

[00163] Methods of performing motility assays are well known to those of skill in the art. (See e.g., Hall, et al. (1996), Biophys. J., 71: 3467-3476, Turner et al., 1996, AnaL Biochem. 242 (1):20-5; Gittes et al., 1996, Biophys. J. 70(l): 418-29; Shirakawa et al., 1995, J. Exp. BioL 198: 1809-15; Winkelmann et al., 1995, Biophys. J. 68: 2444-53; Winkelmann et al., 1995, Biophys. J. 68: 72S.)

[00164] Methods known in the art for determining ATPase hydrolysis activity also can be used. Suitably, solution based assays are utilized. U.S. Patent 6,410,254, hereby incorporated by reference in its entirety, describes such assays. Alternatively, conventional methods are used. For example, P_i release from kinesin can be quantified. In one embodiment, the ATPase hydrolysis activity assay utilizes 0.3 M PCA (perchloric acid) and malachite green reagent (8.27 mM sodium molybdate II, 0.33 mM malachite green oxalate, and 0.8 mM Triton X-1 00). To perform the assay, 10 μL of the reaction mixture is quenched in 90 μL of cold 0.3 M PCA. Phosphate standards are used so data can be converted to mM inorganic phosphate released. When all reactions and standards have been quenched in PCA, 100 μL of malachite green reagent is added to the relevant wells in e.g., a microtiter plate. The mixture is developed for 10-15 minutes and the plate is read at an absorbance of 650 nm. If phosphate standards were used, absorbance readings can be converted to mM P_i and

plotted over time. Additionally, ATPase assays known in the art include the luciferase assay.

[00165] ATPase activity of kinesin motor domains also can be used to monitor the effects of agents and are well known to those skilled in the art. In one embodiment ATPase assays of kinesin are performed in the absence of microtubules. In another embodiment, the ATPase assays are performed in the presence of microtubules. Different types of agents can be detected in the above assays. In a one embodiment, the effect of an agent is independent of the concentration of microtubules and ATP. In another embodiment, the effect of the agents on kinesin ATPase can be decreased by increasing the concentrations of ATP, microtubules or both. In yet another embodiment, the effect of the agent is increased by increasing concentrations of ATP, microtubules or both.

[00166] Compounds that inhibit the biochemical activity of KSP in vitro may then be screened in vivo. In vivo screening methods include assays of cell cycle distribution, cell viability, or the presence, morphology, activity, distribution, or number of mitotic spindles. Methods for monitoring cell cycle distribution of a cell population, for example, by flow cytometry, are well known to those skilled in the art, as are methods for determining cell viability. See for example, U.S. Patent 6,437,115, hereby incorporated by reference in its entirety. Microscopic methods for monitoring spindle formation and malformation are well known to those of skill in the art (see, e.g., Whitehead and Rattner (1998), J. Cell Sci. 111:2551-61; Galgio et al, (1996) J. Cell Biol., 135:399-414), each incorporated herein by reference in its entirety.

[00167] The compounds of the invention inhibit the KSP kinesin. One measure of inhibition is IC_{50}, defined as the concentration of the compound at which the activity of KSP is decreased by fifty percent relative to a control. Preferred compounds have IC_{50}'s of less than about 1 mM, with preferred embodiments having IC_{50}'s of less than about 100 μM, with more preferred embodiments having IC_{50}'s of less than about 10 μM, with particularly preferred embodiments having IC_{50}'s of less than about 1 μM, and especially preferred embodiments having IC_{50}'s of less than about 100 nM, and with the most preferred embodiments having IC_{50}'s of less than about 10 nM. Measurement of IC_{50} is done using an ATPase assay such as described herein.

[00168] Another measure of inhibition is K_i. For compounds with IC_{50}'s less

than 1 μM, the K_i or K_d is defined as the dissociation rate constant for the interaction of the compounds described herein with KSP. Preferred compounds have K_i's of less than about 100 μM, with preferred embodiments having K_i's of less than about 10 μM, and particularly preferred embodiments having K_i's of less than about 1 μM and especially preferred embodiments having K_i's of less than about 100 nM, and with the most preferred embodiments having K_i's of less than about 10 nM.

[00169] The K_i for a compound is determined from the IC_{50} based on three assumptions and the Michaelis-Menten equation. First, only one compound molecule binds to the enzyme and there is no cooperativity. Second, the concentrations of active enzyme and the compound tested are known (i.e., there are no significant amounts of impurities or inactive forms in the preparations). Third, the enzymatic rate of the enzyme-inhibitor complex is zero. The rate (i.e., compound concentration) data are fitted to the equation:

$$V = V_{max} E_0 \left[I - \frac{(E_0 + I_0 + Kd) - \sqrt{(E_0 + I_0 + Kd)^2 - 4 E_0 I_0}}{2E_0} \right]$$

where V is the observed rate, V_{max} is the rate of the free enzyme, I_0 is the inhibitor concentration, E_0 is the enzyme concentration, and K_d is the dissociation constant of the enzyme-inhibitor complex.

[00170] Another measure of inhibition is GI_{50}, defined as the concentration of the compound that results in a decrease in the rate of cell growth by fifty percent. Preferred compounds have GI_{50}'s of less than about 1 mM; those having a GI_{50} of less than about 20 μM are more preferred; those having a GI_{50} of less than about 10 μM more so; those having a GI_{50} of less than about 1 μM more so; those having a GI_{50} of less than about 100 nM more so; and those having a GI_{50} of less than about 10 nM even more so. Measurement of GI_{50} is done using a cell proliferation assay such as described herein. Compounds of this class were found to inhibit cell proliferation.

[00171] In vitro potency of small molecule inhibitors is determined, for example, by assaying human ovarian cancer cells (SKOV3) for viability following a 72-hour exposure to a 9-point dilution series of compound. Cell viability is determined by measuring the absorbance of formazon, a product formed by the bioreduction of MTS/PMS, a commercially available reagent. Each point on the dose-

response curve is calculated as a percent of untreated control cells at 72 hours minus background absorption (complete cell kill).

[00172] Anti-proliferative compounds that have been successfully applied in the clinic to treatment of cancer (cancer chemotherapeutics) have GI_{50}'s that vary greatly. For example, in A549 cells, paclitaxel GI_{50} is 4 nM, doxorubicin is 63 nM, 5-fluorouracil is 1 μM, and hydroxyurea is 500 μM (data provided by National Cancer Institute, Developmental Therapeutic Program, http://dtp.nci.nih.gov/). Therefore, compounds that inhibit cellular proliferation, irrespective of the concentration demonstrating inhibition, have potential clinical usefulness.

[00173] To employ the compounds of the invention in a method of screening for compounds that bind to KSP kinesin, the KSP is bound to a support, and a compound of the invention is added to the assay. Alternatively, the compound of the invention is bound to the support and KSP is added. Classes of compounds among which novel binding agents may be sought include specific antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc. Of particular interest are screening assays for candidate agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, functional assays (phosphorylation assays, etc.) and the like.

[00174] The determination of the binding of the compound of the invention to KSP may be done in a number of ways. In one embodiment, the compound is labeled, for example, with a fluorescent or radioactive moiety, and binding is determined directly. For example, this may be done by attaching all or a portion of KSP to a solid support, adding a labeled test compound (for example a compound of the invention in which at least one atom has been replaced by a detectable isotope), washing off excess reagent, and determining whether the amount of the label is that present on the solid support.

[00175] By "labeled" herein is meant that the compound is either directly or indirectly labeled with a label which provides a detectable signal, e.g., radioisotope, fluorescent tag, enzyme, antibodies, particles such as magnetic particles, chemiluminescent tag, or specific binding molecules, etc. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the

specific binding members, the complementary member would normally be labeled with a molecule which provides for detection, in accordance with known procedures, as outlined above. The label can directly or indirectly provide a detectable signal.

[00176] In some embodiments, only one of the components is labeled. For example, the kinesin proteins may be labeled at tyrosine positions using ^{125}I, or with fluorophores. Alternatively, more than one component may be labeled with different labels; using ^{125}I for the proteins, for example, and a fluorophor for the antimitotic agents.

[00177] The compounds of the invention may also be used as competitors to screen for additional drug candidates. "Candidate agent" or "drug candidate" or grammatical equivalents as used herein describe any molecule, e.g., protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactivity. They may be capable of directly or indirectly altering the cellular proliferation phenotype or the expression of a cellular proliferation sequence, including both nucleic acid sequences and protein sequences. In other cases, alteration of cellular proliferation protein binding and/or activity is screened. Screens of this sort may be performed either in the presence or absence of microtubules. In the case where protein binding or activity is screened, particular embodiments exclude molecules already known to bind to that particular protein, for example, polymer structures such as microtubules, and energy sources such as ATP. Particular embodiments of assays herein include candidate agents which do not bind the cellular proliferation protein in its endogenous native state termed herein as "exogenous" agents. In another embodiment, exogenous agents further exclude antibodies to KSP.

[00178] Candidate agents can encompass numerous chemical classes, though typically they are organic molecules, preferably they are small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding and lipophilic binding, and typically include at least an amine, carbonyl-, hydroxyl-, ether, or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids,

purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[00179] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, and/or amidification to produce structural analogs.

[00180] Competitive screening assays may be done by combining KSP and a drug candidate in a first sample. A second sample comprises a compound of the present invention, KSP and a drug candidate. This may be performed in either the presence or absence of microtubules. The binding of the drug candidate is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of a drug candidate capable of binding to KSP and potentially inhibiting its activity. That is, if the binding of the drug candidate is different in the second sample relative to the first sample, the drug candidate is capable of binding to KSP.

[00181] In a particular embodiment, the binding of the candidate agent to KSP is determined through the use of competitive binding assays. In this embodiment, the competitor is a binding moiety known to bind to KSP, such as an antibody, peptide, binding partner, ligand, etc. Under certain circumstances, there may be competitive binding as between the candidate agent and the binding moiety, with the binding moiety displacing the candidate agent.

[00182] In one embodiment, the candidate agent is labeled. Either the candidate agent, or the competitor, or both, is added first to KSP for a time sufficient to allow binding, if present. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4 and 40°C.

[00183] Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high throughput screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The

second component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

[00184] In another embodiment, the competitor is added first, followed by the candidate agent. Displacement of the competitor is an indication the candidate agent is binding to KSP and thus is capable of binding to, and potentially inhibiting, the activity of KSP. In this embodiment, either component can be labeled. Thus, for example, if the competitor is labeled, the presence of label in the wash solution indicates displacement by the agent. Alternatively, if the candidate agent is labeled, the presence of the label on the support indicates displacement.

[00185] In an alternative embodiment, the candidate agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate the candidate agent is bound to KSP with a higher affinity. Thus, if the candidate agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate the candidate agent is capable of binding to KSP.

[00186] Inhibition is tested by screening for candidate agents capable of inhibiting the activity of KSP comprising the steps of combining a candidate agent with KSP, as above, and determining an alteration in the biological activity of KSP. Thus, in this embodiment, the candidate agent should both bind to KSP (although this may not be necessary), and alter its biological or biochemical activity as defined herein. The methods include both in vitro screening methods and in vivo screening of cells for alterations in cell cycle distribution, cell viability, or for the presence, morpohology, activity, distribution, or amount of mitotic spindles, as are generally outlined above.

[00187] Alternatively, differential screening may be used to identify drug candidates that bind to the native KSP, but cannot bind to modified KSP.

[00188] Positive controls and negative controls may be used in the assays. Suitably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent determined. For example, where a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of bound compound.

[00189] A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in any order that provides for the requisite binding.

Administration

[00190] Accordingly, the compounds of the invention are administered to cells. By "administered" herein is meant administration of a therapeutically effective dose of a compound of the invention to a cell either in cell culture or in a patient. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art, adjustments for systemic versus localized delivery, age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art. By "cells" herein is meant any cell in which mitosis or meiosis can be altered.

[00191] A "patient" for the purposes of the present invention includes both humans and other animals, particularly mammals, and other organisms. Thus the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the patient is a mammal, and in the most preferred embodiment the patient is human.

[00192] Compounds of the invention having the desired pharmacological activity may be administered, preferably as a pharmaceutically acceptable composition comprising an pharmaceutical excipient, to a patient, as described herein. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways as discussed below. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt.%.

[00193] The agents may be administered alone or in combination with other treatments, i.e., radiation, or other chemotherapeutic agents such as the taxane class of

agents that appear to act on microtubule formation or the camptothecin class of topoisomerase I inhibitors. When used, other chemotherapeutic agents may be administered before, concurrently, or after administration of a compound of the present invention. In one aspect of the invention, a compound of the present invention is co-administered with one or more other chemotherapeutic agents. By "co-administer" it is meant that the present compounds are administered to a patient such that the present compounds as well as the co-administered compound may be found in the patient's bloodstream at the same time, regardless when the compounds are actually administered, including simultaneously.

[00194] The administration of the compounds and compositions of the present invention can be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in the treatment of wounds and inflammation, the compound or composition may be directly applied as a solution or spray.

[00195] Pharmaceutical dosage forms include a compound of formula I or a pharmaceutically acceptable salt, solvate, or solvate of a salt thereof, and one or more pharmaceutical excipients. As is known in the art, pharmaceutical excipients are secondary ingredients which function to enable or enhance the delivery of a drug or medicine in a variety of dosage forms (e.g.: oral forms such as tablets, capsules, and liquids; topical forms such as dermal, opthalmic, and otic forms; suppositories; injectables; respiratory forms and the like). Pharmaceutical excipients include inert or inactive ingredients, synergists or chemicals that substantively contribute to the medicinal effects of the active ingredient. For example, pharmaceutical excipients may function to improve flow characteristics, product uniformity, stability, taste, or appearance, to ease handling and administration of dose, for convenience of use, or to control bioavailability. While pharmaceutical excipients are commonly described as being inert or inactive, it is appreciated in the art that there is a relationship between the properties of the pharmaceutical excipients and the dosage forms containing them.

[00196] Pharmaceutical excipients suitable for use as carriers or diluents are well known in the art, and may be used in a variety of formulations. See, e.g., Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, Editor, Mack Publishing Company (1990); Remington: The Science and Practice of Pharmacy, 20th

Edition, A. R. Gennaro, Editor, Lippincott Williams & Wilkins (2000); Handbook of Pharmaceutical Excipients, 3rd Edition, A. H. Kibbe, Editor, American Pharmaceutical Association, and Pharmaceutical Press (2000); and Handbook of Pharmaceutical Additives, compiled by Michael and Irene Ash,Gower (1995), each of which is incorporated herein by reference for all purposes.

[00197] Oral solid dosage forms such as tablets will typically comprise one or more pharmaceutical excipients, which may for example help impart satisfactory processing and compression characteristics, or provide additional desirable physical characteristics to the tablet. Such pharmaceutical excipients may be selected from diluents, binders, glidants, lubricants, disintegrants, colors, flavors, sweetening agents, polymers, waxes or other solubility-retarding materials.

[00198] Compositions for intravenous administration will generally comprise intravenous fluids, i.e., sterile solutions of simple chemicals such as sugars, amino acids or electrolytes, which can be easily carried by the circulatory system and assimilated. Such fluids are prepared with water for injection USP.

[00199] Dosage forms for parenteral administration will generally comprise fluids, particularly intravenous fluids, i.e., sterile solutions of simple chemicals such as sugars, amino acids or electrolytes, which can be easily carried by the circulatory system and assimilated. Such fluids are typically prepared with water for injection USP. Fluids used commonly for intravenous (IV) use are disclosed in Remington, The Science and Practice of Pharmacy [full citation previously provided], and include:

- alcohol, e.g., 5% alcohol (e.g., in dextrose and water ("D/W") or D/W in normal saline solution ("NSS"), including in 5% dextrose and water ("D5/W"), or D5/W in NSS);
- synthetic amino acid such as Aminosyn, FreAmine, Travasol, e.g., 3.5 or 7; 8.5; 3.5, 5.5 or 8.5 % respectively;
- ammonium chloride e.g., 2.14%;
- dextran 40, in NSS e.g., 10% or in D5/W e.g., 10%;
- dextran 70, in NSS e.g., 6% or in D5/W e.g., 6%;
- dextrose (glucose, D5/W) e.g., 2.5-50%;
- dextrose and sodium chloride e.g., 5-20% dextrose and 0.22-0.9% NaCl;

- lactated Ringer's (Hartmann's) e.g., NaCl 0.6%, KCl 0.03%, $CaCl_2$ 0.02%;
- lactate 0.3%;
- mannitol e.g., 5%, optionally in combination with dextrose e.g., 10% or NaCl e.g., 15 or 20%;
- multiple electrolyte solutions with varying combinations of electrolytes, dextrose, fructose , invert sugar Ringer's e.g., NaCl 0.86%, KCl 0.03%, $CaCl_2$ 0.033%;
- sodium bicarbonate e.g., 5%;
- sodium chloride e.g., 0.45, 0.9, 3, or 5%;
- sodium lactate e.g., 1/6 M; and
- sterile water for injection

The pH of such IV fluids may vary, and will typically be from 3.5 to 8 as known in the art.

[00200] The compounds, pharmaceutically acceptable salts and solvates of the invention can be administered alone or in combination with other treatments, i.e., radiation, or other therapeutic agents, such as the taxane class of agents that appear to act on microtubule formation or the camptothecin class of topoisomerase I inhibitors. When so-used, other therapeutic agents can be administered before, concurrently (whether in separate dosage forms or in a combined dosage form), or after administration of an active agent of the present invention.

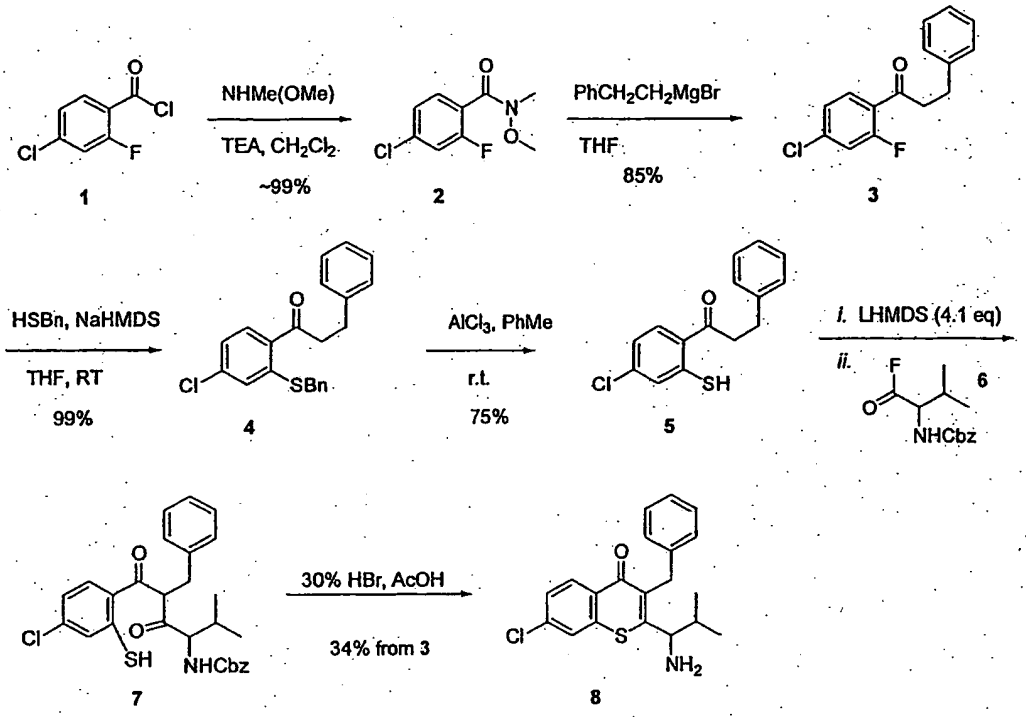
[00201] The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

EXAMPLES

[00202] All anhydrous solvents were purchased from Aldrich Chemical Company in SureSeal® containers. Most reagents were purchased from Aldrich Chemical Company.

Example 1

Synthesis of Compounds



[00203] *N,N*-Dimethylhydroxylamine hydrochloride (11.55 g, 125 mmol) was added to a 0 °C solution of 1 (20 g, 104 mmol), triethylamine (43 mL, 312 mmol), and CH_2Cl_2 (200 mL). After 15 minutes, the reaction mixture was quenched with H_2O (100 mL) and the layers were separated. The organic layer was washed with brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to provide 22.6 g (~99%) of 2, which was used without further purification.

[00204] (2-Bromoethyl)benzene (28.0 mL, 208 mmol), magnesium turnings (5.6 g, 229 mmol), and THF (300 mL) were mixed in a 1L round-bottom flask equipped with a reflux condenser at 23°C under a N_2 atmosphere. After ~10 minutes

the reaction mixture begins to exotherm and the reaction mixture was allowed to progress to reflux. After 1.5 hour, the Grignard reaction was complete and the solution had cooled to 23°C. A solution of **2** (~22.6 g, ~104 mmol) and THF (100 mL) was added via cannula to the 20°C solution of the phenethyl magnesiumbromide. The temperature was monitored by internal thermometer and was not allowed to exceed ~40 C. After 3 h at 23°C, the reaction mixture was quenched by pouring into 1 N HCl (300 mL). The layers were separated and the organic layer was washed with brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by flash column chromatography (100% hexanes; 50:1 hexanes:EtOAc; 25:1 hexanes:EtOAc) to yield 23.2 g (85%) of **3**. LRMS (MH) *m/z* 263.2.

[00205] Sodium hydride (1.36 g, 34 mmol) was added to a 23 C solution of α-toluenetiol (3.66 mL, 30.9 mmol) in DMF (150 mL). After 30 minutes, ketone **3** (8.1 g, 30.9 mmol) was added in one portion. After 1 hour at 23°C, the reaction mixture was quenched by pouring into 1 N HCl (100 mL) and EtOAc (150 mL). The layers were separated and the organic layer was washed with brine (3 x 150 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to provide 11.3 g (~99%) of **4**, which was used without further purification.

[00206] Aluminum trichloride (12.4 g, 92.7 mmol) was added to a 23°C solution of ketone **4** (12.3 g, 33.5 mmol) and PhMe (160 mL). After 1 hour at 23°C, the reaction mixture was quenched by pouring into H_2O (200 mL) and EtOAc (150 mL). The layers were separated and the organic layer was washed with brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by flash column chromatography (30:1 hexanes:EtOAc) to yield 9.27 g (85%) of **5**. LRMS (MH) *m/z* 277.1.

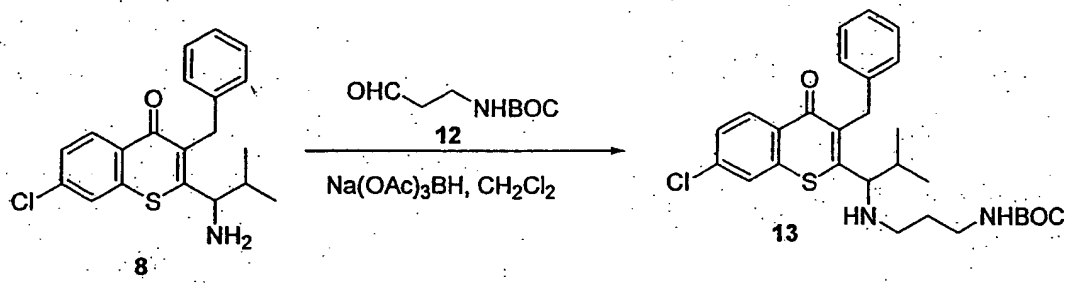
[00207] Lithium bis(trimethylsilyl)amide (LHMDS, 1.0 M in THF, 15.0 mL, 4.1 equiv) was added to a –78°C solution of ketone **5** (1.015 g, 3.66 mmol) and THF (15 mL). After the addition was complete the resulting solution was maintained at –78°C for 1 hour. The reaction solution was then warmed to 0°C for 2 hours. The reaction solution was then cooled to –78°C. A solution of acid fluoride **6** (1.019 g, 4.03 mmol) and THF (10 mL) was added drop-wise via syringe to the reaction solution. After 15 minutes at –78°C, the reaction solution was warmed to 23°C. After 2 hours, the reaction solution was quenched with 1 N HCl (20 mL). The layers were

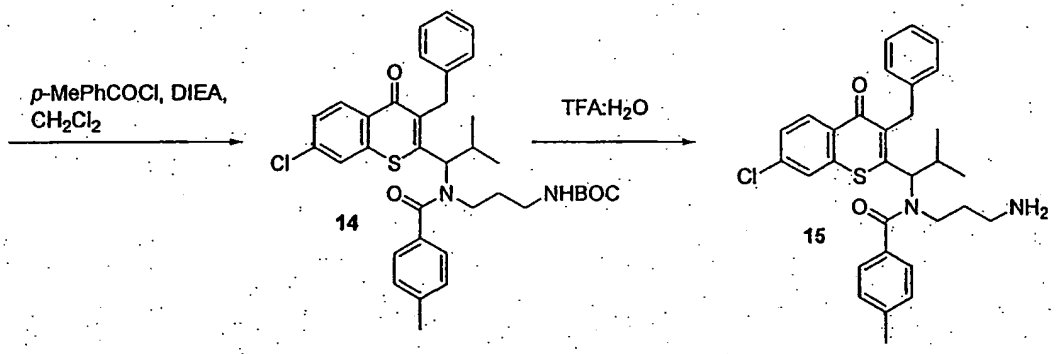
separated and the organic layer was washed with brine (20 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The resulting residue was used without further purification.

[00208] A solution of crude 7 (~3.66 mmol) and 30% HBr in AcOH (30 mL) was maintained at 23°C for 1 hour. The reaction solution was then concentrated. The resulting residue was purified by flash column chromatography (2:1 hexanes:EtOAc) to yield 441 mg (34% from 5) of 8. LRMS (MH) *m/z* 358.1.

[00209] Cbz-Valine-F was prepared according to literature procedure.[1]

Example 2





[00210] Thiochromenone 8 (420 mg, 1.24 mmol), aldehyde 12 (280 mg, 1.6 mmol), $NaCN(OAc)_3BH$ (790 mg, 3.7 mmol), and CH_2Cl_2 (4.1 mL) is maintained at 23°C for 3 h. The reaction mixture is diluted with EtOAc (20 mL) and washed with 1

1 J. Org. Chem., 56(8), 2611-14; 1991

N NaOH (5 mL) and brine (5 mL). The organic layer is dried ($MgSO_4$), filtered, and concentrated. The resulting residue is purified by flash column chromatography (5:1 hexanes:EtOAc; 3:1 hexanes:EtOAc) to yield 460 mg (75%) of **13** as a viscous oil.

[00211] To a solution of thiochromenone **13** (1.3 g, 2.6 mmol), diisoproylethylamine (DIEA, 1.8 mL), and CH_2Cl_2 (7.5 mL) at 23°C is added *p*-toluoyl chloride (0.7 mL, 5.22 mmol). After 2.5 h, the reaction mixture is diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL) and brine (20 mL). The organic layer is dried ($MgSO_4$), filtered, and concentrated. The resulting residue is purified by flash column chromatography (3:1 hexanes:EtOAc) to yield 1.43 g (89%) of **14** as a colorless oil.

[00212] Thiochromenone **14** (1.43 g, 2.32 mmol) and TFA:H_2O (97.5:2.5, 30 mL) is maintained at 23°C for 1h. The reaction mixture is concentrated. The residue is dissolved in EtOAc (100 mL) and washed with 1 N NaOH (25 mL) and brine (25 mL). The organic layer is dried ($MgSO_4$), filtered, and concentrated to provide a white solid which was deemed >95% pure by ^1H NMR and LCMS analysis.

Example 3

Induction of Mitotic Arrest in Cell Populations Treated with a KSP Inhibitor

[00213] FACS analysis to determine cell cycle stage by measuring DNA content is performed as follows. Skov-3 cells (human ovarian cancer) are split 1:10 for plating in 10cm dishes and grown to subconfluence with RPMI 1640 medium containing 5% fetal bovine serum (FBS). The cells are then treated with either 10nM paclitaxel, 400nM test compound, 200nM test compound, or 0.25% DMSO (vehicle for compounds) for 24 hours. A well known anti-mitotic agent, such as placitaxel, is used as a positive cotnrol. Cells are then rinsed off the plates with PBS containing 5mM EDTA, pelleted, washed once in PBS containing 1% FCS, and then fixed overnight in 85% ethanol at 4°C. Before analysis, the cells are pelleted, washed once, and stained in a solution of 10μg propidium iodide and 250μg of ribonuclease (RNAse) A per milliliter at 37°C for half an hour. Flow cytometry analysis is performed on a Becton-Dickinson FACScan, and data from 10,000 cells per sample is analyzed with Modfit software.

Example 4

Monopolar Spindle Formation following Application of a KSP Inhibitor

[00214] To determine the nature of G2/M accumulation, human tumor cell lines Skov-3 (ovarian), HeLa (cervical), and A549 (lung) are plated in 96-well plates at densities of 4,000 cells per well (SKOV-3 & HeLa) or 8,000 cells per well (A549), allowed to adhere for 24 hours, and treated with various concentrations of the test compounds for 24 hours. Cells are fixed in 4% formaldehyde and stained with anti-tubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which stains DNA). The cells can be visually inspected to assess the effects of the test compounds. For example, microinjection of anti-KSP antibodies causes mitotic arrest with arrested cells displaying monopolar spindles.

Example 5

Inhibition of Cellular Proliferation in Tumor Cell Lines Treated with KSP Inhibitors.

[00215] Cells are plated in 96-well plates at densities from 1000-2500 cells/well (depending on the cell line) and allowed to adhere/grow for 24 hours. They are then treated with various concentrations of test compound for 48 hours. The time at which compounds are added is considered T_0. A tetrazolium-based assay using the reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (U.S. Patent No. 5,185,450) (see Promega product catalog #G3580, CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay) is used to determine the number of viable cells at T_0 and the number of cells remaining after 48 hours compound exposure. The number of cells remaining after 48 hours is compared to the number of viable cells at the time of test compound addition, allowing for calculation of growth inhibition. The growth over 48 hours of cells in control wells treated with vehicle only (0.25% DMSO) is considered 100% growth and the growth of cells in wells with compounds is compared to this. Active KSP inhibitors inhibit cell proliferation in one or more human tumor cell lines of the following tumor types: lung (NCI-H460, A549), breast (MDA-MB-231, MCF-7, MCF-7/ADR-RES), colon (HT29, HCT15), ovarian (SKOV-3, OVCAR-3), leukemia (HL-60(TB), K-562),

central nervous system (SF-268), renal (A498), osteosarcoma (U2-OS), and cervical (HeLa), and mouse tumor line (B16, melanoma).

[00216] Calculation Of GI_{50}: A GI_{50} is calculated by plotting the concentration of compound in μM vs the percentage of cell growth of cell growth in treated wells. The GI_{50} calculated for the compounds is the estimated concentration at which growth is inhibited by 50% compared to control, i.e., the concentration at which:

$$100 \times [(Treated_{48} - T_0) / (Control_{48} - T_0)] = 50.$$

All concentrations of compounds are tested in duplicate and controls are averaged over 12 wells. A very similar 96-well plate layout and GI_{50} calculation scheme is used by the National Cancer Institute (see Monks, et al., J. Natl. Cancer Inst. 83:757-766 (1991)). However, the method by which the National Cancer Institute quantitates cell number does not use MTS, but instead employs alternative methods.

[00217] Calculation Of IC_{50}: Measurement of a compound's IC_{50} for KSP activity uses an ATPase assay. The following solutions are used: Solution 1 consists of 3 mM phosphoenolpyruvate potassium salt (Sigma P-7127), 2 mM ATP (Sigma A-3377), 1 mM IDTT (Sigma D-9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT400301), and 1 mM EGTA (Sigma E3889). Solution 2 consists of 1 mM NADH (Sigma N8129), 0.2 mg/ml BSA (Sigma A7906), pyruvate kinase 7U/ml, L-lactate dehydrogenase 10 U/ml (Sigma P0294), 100 nM KSP motor domain, 50 μg/ml microtubules, 1 mM DTT (Sigma D9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT4003-01), and 1 mM EGTA (Sigma E3889). Serial dilutions (8-12 two-fold dilutions) of the composition are made in a 96-well microtiter plate (Corning Costar 3695) using Solution 1. Following serial dilution each well has 50 μl of Solution 1. The reaction is started by adding 50 μl of Solution 2 to each well. This can be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC_{50} determination the data acquired is fit by the following four parameter equation using a

nonlinear fitting program (e.g., Grafit 4):

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^{s}} + \text{Background}$$

where y is the observed rate and x the compound concentration.

Example 6

Inhibition of Cellular Viability in Tumor Cell Lines Treated with KSP Inhibitors.

Materials and Solutions:

- Cells: SKOV3, Ovarian Cancer (human).
- Media: Phenol Red Free RPMI + 5% Fetal Bovine Serum + 2mM L-glutamine.
- Colorimetric Agent for Determining Cell Viability: Promega MTS tetrazolium compound.
- Control Compound for max cell kill: Topotecan, 1μM.

[00218] Procedure: Day 1 - Cell Plating: Adherent SKOV3 cells are washed with 10mLs of PBS followed by the addition of 2mLs of 0.25% trypsin and incubation for 5 minutes at 37°C. The cells are rinsed from the flask using 8 mL of media (phenol red-free RPMI+ 5%FBS) and transferred to fresh flask. Cell concentration is determined using a Coulter counter and the appropriate volume of cells to achieve 1000 cells/100μL is calculated. 100 μL of media cell suspension (adjusted to 1000 cells/100 μL) is added to all wells of 96-well plates, followed by incubation for 18 to 24 hours at 37°C, 100% humidity, and 5% CO_2, allowing the cells to adhere to the plates.

[00219] Procedure: Day 2 – Compound Addition: To one column of the wells of an autoclaved assay block are added an initial 2.5 μL of test compound(s) at 400X the highest desired concentration. 1.25 μL of 400X (400μM) Topotecan is added to other wells (ODs from these wells are used to subtract out for background absorbance of dead cells and vehicle). 500 μL of media without DMSO are added to

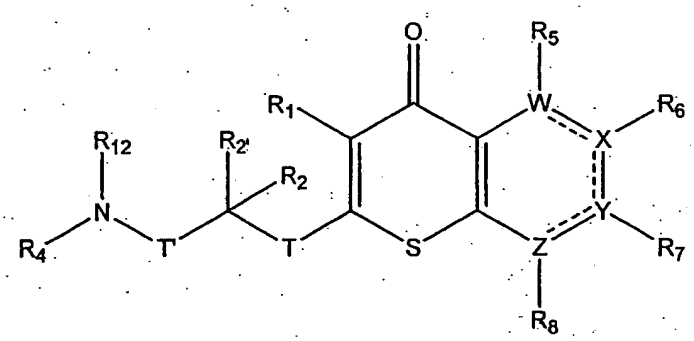
the wells containing test compound, and 250 µL to the Topotecan wells. 250 µL of media + 0.5% DMSO is added to all remaining wells, into which the test compound(s) are serially diluted. By row, compound-containing media is replica plated (in duplicate) from the assay block to the corresponding cell plates. The cell plates are incubated for 72hours at 37°C, 100% humidity, and 5% CO_2.

[00220] Procedure: Day 4 – MTS Addition and OD Reading: The plates are removed from the incubator and 40 µl MTS / PMS is added to each well. Plates are then incubated for 120 minutes at 37°C, 100% humidity, 5%CO_2, followed by reading the ODs at 490nm after a 5 second shaking cycle in a ninety-six well spectrophotometer.

[00221] Data Analysis The normalized % of control (absorbance- background) is calculated and an XLfit is used to generate a dose-response curve from which the concentration of compound required to inhibit viability by 50% is determined.

What is claimed is:

1. A compound selected from the group represented by Formula I:



Formula I

wherein:

W, X, Y, and Z are independently N, C, CH, O, or S; and Z is optionally absent, provided that:

no more than two of W, X, Y, and Z is –N=, and

W, X, or Y can be O or S only when Z is absent;

the dashed lines in the structure depict optional double bonds;

T and T' are independently a covalent bond, -C(O)-, or optionally substituted lower alkylene;

R_1 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

R_2 and $R_{2'}$ are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; or R_2 and $R_{2'}$ taken together form an optionally substituted 3- to 7-membered ring;

R_{12} is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, -C(O)-R_3, and -$S(O)_2$-R_{3a};

R_3 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, $R_{15}O$- and R_{17}-NH-;

R_{3a} is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, and R_{15}-NH-;

R_4 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaralkyl-, and optionally substituted heterocyclyl-;

or R_4 taken together with R_{12}, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring;

or R_4 taken together with R_2 form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring;

R_5, R_6, R_7 and R_8 are independently chosen from hydrogen, acyl, optionally substituted alkyl-, optionally substituted alkoxy, halogen, hydroxyl, nitro, cyano, optionally substituted amino, alkylsulfonyl-, alkylsulfonamido-, alkylthio-, carboxyalkyl-, aminocarbonyl-, optionally substituted aryl and optionally substituted heteroaryl-, provided that R_5, R_6, R_7 and R_8 is absent where W, X, Y, or Z, respectively, is –N=, O, S or absent;

R_{15} is chosen from optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, and optionally substituted heteroaralkyl-; and

R_{17} is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted hetero-aralkyl-,

or a pharmaceutically acceptable salt or solvate thereof.

2. The compound of Claim 1 comprising one or more of the following:

one or both of T and T' is a covalent bond;

W, X, Y and Z are independently –C= or –N=;

R_1 is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted phenyl-C_1-C_4-alkyl-, optionally substituted heteroaryl- C_1-C_4-alkyl-, optionally substituted naphthalenylmethyl, optionally substituted phenyl, or naphthyl;

R_2 is optionally substituted C_1-C_4 alkyl;

$R_{2'}$ is hydrogen;

R_{12} is –C(O)R_3;

R_3 is selected from optionally substituted C_1-C_8 alkyl, optionally substituted aryl-C_1-C_4-alkyl-, optionally substituted heteroaryl-C_1-C_4-alkyl-, optionally substituted heteroaryl, optionally substituted aryl, R_{15}O- and R_{17}-NH-;

R_{15} is chosen from optionally substituted C_1-C_8 alkyl and optionally substituted aryl. ;

R_{17} is chosen from hydrogen, C_1-C_4 alkyl; cyclohexyl; phenyl; and phenyl substituted with halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, or C_1-C_4 alkylthio;

R_4 is chosen from hydrogen, C_1-C_4 alkyl; cyclohexyl; phenyl substituted with hydroxyl, C_1-C_4 alkoxy or C_1-C_4 alkyl; benzyl; heteroarylmethyl-; heteroarylethyl-; heteroarylpropyl-; and R_{16}-alkylene-;

R_{16} is hydroxyl, di(C_1-C_4 alkyl)amino-, (C_1-C_4 alkyl)amino-, amino, C_1-C_4 alkoxy-, or N-heterocyclyl-, particularly pyrrolidino, piperidino or imidazolyl.; and

R_5, R_6, R_7 and R_8 are independently methoxy, hydrogen, cyano, or halo, provided that R_5, R_6, R_7 and R_8 is absent where W, X, Y, or Z, respectively, is –N=.

3. The compound of Claim 2 comprising one or more of the following:

both T and T' are covalent bonds;

W, X, Y and Z are C;

R_1 is optionally substituted phenyl-C_1-C_4-alkyl- or optionally substituted heteroaryl-C_1-C_4-alkyl-.

R_2 is ethyl or propyl;

R_3 is optionally substituted C_1-C_8 alkyl, optionally substituted heteroaryl, or optionally substituted aryl;

R_4 is R_{16}-alkylene-;

R_{16} is amino, C_1-C_4 alkylamino-, di(C_1-C_4 alkyl)amino-, C_1-C_4 alkoxy-, hydroxyl, or N-heterocyclyl;

R_5 is amino, alkylamino, trifluoromethyl, hydrogen or halo;

R_6 is hydrogen, alkyl, or halo;

R_7 is hydrogen, halo, alkyl, alkoxy, cyano, or trifluoromethyl; and

R_8 is hydrogen or halo.

4. The compound of Claim 3 comprising one or more of the following:

R_1 is naphthyl, phenyl, bromophenyl, chlorophenyl, methoxyphenyl, ethoxyphenyl, tolyl, dimethylphenyl, chorofluorophenyl, methylchlorophenyl, ethylphenyl, phenethyl, benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, hydroxybenzyl, dichlorobenzyl, dimethoxybenzyl, or naphthalenylmethyl;

R_2 is *i*-propyl;

R_3 is tolyl, halophenyl, halomethylphenyl, hydroxymethylphenyl, methylenedioxyphenyl, formylphenyl or cyanophenyl;

R_4 is R_{16}-alkylene-;

R_{16} is amino;

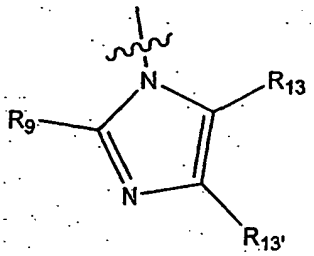
R_5, R_6, and R_8 are hydrogen; and

R_7 is cyano, methoxy or halogen.

5. The compound of claim 4 wherein R_1 is benzyl, cyanobenzyl, methoxybenzyl, or naphthalenylmethyl.

6. The compound of claim 5 wherein R_1 is benzyl.

7. The compound of claim 1, wherein R_4 taken together with R_{12} and the nitrogen to which they are bound, forms an optionally substituted imidazolinyl ring of the formula:



wherein

R_9 is chosen from hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, optionally substituted aryl-C_1-C_4-alkyl -, optionally substituted heteroaryl-C_1-C_4-alkyl -, optionally substituted aryl-C_1-C_4-alkoxy -, optionally substituted heteroaryl-C_1-C_4-alkoxy -, optionally substituted heteroaryl-; and

R_{13} and $R_{13'}$ are independently hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, or optionally substituted aryl-C_1-C_4-alkyl - (especially optionally substituted alkyl).

8. The compound of claim 7 comprising one or more of the following:

R_9 is phenyl substituted with C_1-C_4-alkyl, C_1-C_4-alkoxy-, and/or halo; phenyl; or benzyl;

R_{13} is hydrogen; and

$R_{13'}$ is substituted C_1-C_4 alkyl.

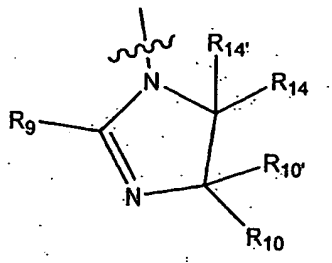
9. The compound of claim 8 comprising one or more of the following:

R_9 is tolyl; halophenyl; or halomethylphenyl;

R_{13} is hydrogen; and

$R_{13'}$ is aminomethyl, aminoethyl, aminopropyl, acetylamino-methyl, acetylaminoethyl, benzyloxycarbonylamino-methyl or benzyloxycarbonylamino-ethyl.

10. The compound of claim 1 wherein R_{12} taken together with R_4 forms an optionally substituted imidazolinyl ring of the formula:



wherein

R_9 is chosen from hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, optionally substituted aryl-C_1-C_4-alkyl -, and optionally substituted heteroaryl-; and

R_{10}, $R_{10'}$, R_{14}, and $R_{14'}$ are independently chosen from hydrogen, optionally substituted C_1-C_8 alkyl, optionally substituted aryl, and optionally substituted aryl-C_1-C_4-alkyl -.

11. The compound of claim 10 comprising one or more of the following:

R_9 is methylenedioxyphenyl; phenyl; phenyl substituted with C_1-C_4 alkyl, C_1-C_4 alkoxy, and/or halo; or benzyl; and

R_{10}, $R_{10'}$, $R_{14'}$, and R_{14} are independently hydrogen or optionally substituted alkyl.

12. The compound of claim 11 comprising one or more of the following:

R_9 is methylenedioxyphenyl-; phenyl; or phenyl substituted with methoxy, halo and/or methyl;

R_{10} and $R_{10'}$ are independently selected from the group consisting of hydrogen or optionally substituted C_1-C_4 alkyl; and

$R_{14'}$ and R_{14} are hydrogen.

13. The compound of any of the above claims wherein the stereogenic center to which R_2 and $R_{2'}$ are attached is of the R configuration.

14. A pharmaceutical formulation comprising a pharmaceutically acceptable excipient and an effective amount of a compound of any of Claims 1-12.

15. A method of treatment comprising administering an effective amount of a compound of any of Claims 1-12 to a patient suffering from a cellular proliferative disease.

16. The method of Claim 15 wherein the cellular proliferative disease is cancer, hyperplasia, restenosis, cardiac hypertrophy, an immune disorder or inflammation.

17. A method of treatment for a cellular proliferative disease comprising administering to a patient suffering therefrom a compound of Claim 1 in an amount sufficient to modulate KSP kinesin activity in cells affected with the disease.

18. A kit comprising a compound of any of Claims 1-12 and a package insert or other labeling including directions for treating a cellular proliferative disease by administering an effective amount of said compound.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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60/410,743 13 September 2002 (13.09.2002) US

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WO 2004/024086 A2

(54) Title: COMPOUNDS, COMPOSITIONS AND METHODS

(57) Abstract: Compounds, compositions and methods useful for treating cellular proliferative diseases and disorders, for example, by modulating the activity of KSP, are disclosed.

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October 13, 2005

Andrew McDonald, Ph.D.
ThinkEquity Partners LLC
600 Montgomery Street
San Francisco, CA 94111

VIA FEDERAL EXPRESS

Cytokinetics, Inc. Patent Application

Dear Andrew:

Further to our letter of July 15, 2005, please find enclosed certain documents for filing in the U.S. Patent and Trademark Office that require your signature.

The enclosed documents are the confidential and proprietary property of Cytokinetics, Inc. Consequently, the enclosed documents should not be copied or disclosed to anyone; however, you may retain copies of the application, the signed Declaration and Power of Attorney, and Assignment of Patent Application provided they are kept confidential and not disclosed to anyone.

Because you are a co-inventor of this application, we require your signature on the enclosed forms. Please review the enclosed copy of the application as filed, and sign and date all the forms as indicated.

Once you have reviewed and signed the enclosed documents at the indicated locations, please return all of the enclosed documents to us using the enclosed prepaid Federal Express envelope and address label.

To comply with U.S. Patent and Trademark Office rules, please let us know of any information and materials, including any prior art, that you are aware of that would be material to the examination of these applications. Information is considered material if there is a substantial likelihood that an Examiner would consider it important in deciding the patentability of the invention. To the extent that such information exists, it should be submitted to the U.S. Patent and Trademark Office within three (3) months of the filing date, or as soon thereafter as possible.

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Because we would like to file these documents as soon as possible, we ask that you please return the signed documents to us at your earliest convenience, and at most by November 11, 2005.

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Lauren L. Stevens, Ph.D.

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LAUREN L. STEVENS, PH.D
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September 28, 2006

Andrew McDonald, Ph.D.
ThinkEquity Partners LLC
600 Montgomery Street
San Francisco, CA 94111

VIA FEDERAL EXPRESS

Cytokinetics, Inc. Patent Application
Our Reference: 09367.0044-00000

Dear Andrew:

We are currently prosecuting an application for Cytokinetics, Inc. in the United States. We have enclosed certain documents for filing in the U.S. Patent and Trademark Office that require your signature.

The enclosed documents are the confidential and proprietary property of Cytokinetics, Inc. Consequently, the enclosed documents should not be copied or disclosed to anyone; however, you may retain copies of the application, the signed Declaration and Power of Attorney, and Assignment of Patent Application provided they are kept confidential and not disclosed to anyone.

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Once you have reviewed and signed the enclosed documents at the indicated locations, please return all of the enclosed documents to us.

To comply with U.S. Patent and Trademark Office rules, please let us know of any information and materials, including any prior art, that you are aware of that would be material to the examination of these applications. Information is considered material if there is a substantial likelihood that an Examiner would consider it important in deciding the patentability of the invention. To the extent that such information exists, it should be submitted to the U.S. Patent and Trademark Office within three (3) months of the filing date, or as soon thereafter as possible.

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Please return the signed documents to us at your earliest convenience, and at most by October 2, 2006.

If you have any questions regarding the enclosed documents please contact me at 650-849-6614.

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September 28, 2006

Andrew McDonald, Ph.D.
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Cytokinetics, Inc. Patent Application
Our Reference: 09367.0044-00000

Dear Andrew:

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If you have any questions regarding the enclosed documents please contact me at 650-849-6614.

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LAUREN L. STEVENS, PH.D
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December 7, 2006

Andrew McDonald, Ph.D.
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Cytokinetics, Inc. Patent Application
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LAUREN L. STEVENS, PH.D
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December 7, 2006

Andrew McDonald, Ph.D.
187 Elsie
San Francisco, CA 94110

VIA FEDERAL EXPRESS

Cytokinetics, Inc. Patent Application
Our Reference: 09367.0044-00000

Dear Andrew:

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Please return the signed documents to us at your earliest convenience, and at most by December 15, 2006.

If you have any questions regarding the enclosed documents please contact me at 650-849-6614.

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Lauren L. Stevens, Ph.D.

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Dear Andrew:

Re: U.S. Patent Application
09367.0044-00000

Please see the attached letter, formal documents, and patent application. If you have any questions, please let us know.

Kind regards,
Linda Phillips

Linda Phillips
Patent Legal Assistant
Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
Stanford Research Park
3300 Hillview Avenue
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Direct: 650.849.6648
Facsimile: 650.849.6666
E-mail: linda.phillips@finnegan.com

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LAUREN L. STEVENS, PH.D
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December 7, 2006

Andrew McDonald, Ph.D.
ThinkEquity Partners LLC
600 Montgomery Street
San Francisco, CA 94111

VIA FEDERAL EXPRESS

Cytokinetics, Inc. Patent Application
Our Reference: 09367.0044-00000

Dear Andrew:

We are currently prosecuting an application for Cytokinetics, Inc. in the United States. We have enclosed certain documents for filing in the U.S. Patent and Trademark Office that require your signature.

The enclosed documents are the confidential and proprietary property of Cytokinetics, Inc. Consequently, the enclosed documents should not be copied or disclosed to anyone; however, you may retain copies of the application, the signed Declaration and Power of Attorney, and Assignment of Patent Application provided they are kept confidential and not disclosed to anyone.

Because you are a co-inventor of this application, we require your signature on the enclosed forms. Please review the enclosed copy of the application as filed, and sign and date all the forms as indicated.

Once you have reviewed and signed the enclosed documents at the indicated locations, please return all of the enclosed documents to us.

To comply with U.S. Patent and Trademark Office rules, please let us know of any information and materials, including any prior art, that you are aware of that would be material to the examination of these applications. Information is considered material if there is a substantial likelihood that an Examiner would consider it important in deciding the patentability of the invention. To the extent that such information exists, it should be submitted to the U.S. Patent and Trademark Office within three (3) months of the filing date, or as soon thereafter as possible.

Please remember that the duty to cite material prior art also extends to prior art that you may subsequently become aware of up to the time of issuance of the U.S. patent. This includes, for example, prior art cited during the prosecution of corresponding foreign applications that would be material to the examination of these applications.

Please return the signed documents to us at your earliest convenience, and at most by December 15, 2006.

If you have any questions regarding the enclosed documents please contact me at 650-849-6614.

Very truly yours,

Lauren L. Stevens, Ph.D.

LLS/lp

Enclosures 1) Patent Application
2) Declaration and Power of Attorney
3) Assignment

This tracking update has been requested by:

Company Name: Finnegan, Henderson

Name: Lauren L. Stevens, Ph.D.

E-mail: linda.phillips@finnegan.com

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Packaging type:	FedEx Envelope
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Lauren L. Stevens, Ph.D.
Finnegan, Henderson
3300 Hillview Ave

Palo Alto, CA 94304

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